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R. E. DORER, Treasurer, 300 Essex Bldg., Norfolk, Va.

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THE AMERICAN MOSQUITO CONTROL ASSOCIATION

ARTICLES

ADDITIONAL OBSERVATIONS ON THE CONTROL OF MOSQUITO LARVAE IN ALASKA WITH DDT¹B. V. TRAVIS,² K. H. APPLEWHITE,³ G. R. FRITH,⁴ AND J. B. GOLDSMITH^{5, 6}

Observations and larval dipping counts were made during the season of 1950 in Alaska to determine the effect on mosquito breeding of residual DDT from treatments in 1948 and 1949. Further studies were also made on the effectiveness of different DDT formulations for the control of mosquito larvae in Alaska. These studies were a continuation of those carried out in 1949 (Travis *et al.* 1950).

RESIDUAL EFFECTIVENESS OF DDT SPRAYS AGAINST LARVAE. In 1948, an area of 25 square miles at Eielson Air Force Base near Fairbanks, Alaska, was sprayed three times with DDT solutions, each at the rate of 0.1 pound of DDT per acre, for control of adult mosquitoes. The size of the area was enlarged to 100 square miles in 1949 and was treated once for larval control and twice for adult control. The original 25-square-mile plot, which was in the center of the larger plot, re-

ceived one additional treatment for adult control. These treatments gave a total dosage of 0.7 pound of DDT in 2 years on the central plot, and 0.3 pound in 1 year on the remainder of the large plot. The sprays were applied with a C-47 airplane and consisted of 20 percent DDT in either 60 percent of Paccosol (an auxiliary aromatic hydrocarbon solvent) and 20 percent of fuel oil, or 28 percent of Velsicol AR-50-G (chiefly methylated naphthalenes) and 52 percent of fuel oil.

The residual effect of the treatments was determined by comparing the larval populations in the treated areas with those in adjacent untreated areas, and also by comparing the populations in 1950 with those of 1949. Larval counts were obtained by random dipping every half mile along transects at right angles to a road through the center of the treated area. Each transect was one-quarter to one-half mile long, and none was located closer than one-half mile to the outer boundary of either plot. From 105 to 223 dips were taken along each transect except in one case where water was scarce and only 57 dips were made. Four transects were checked in adjacent untreated areas and larvae were so numerous that an average of only 70 dips was made per transect. The population was largely late third and early fourth instars at the time the observations were made on May 20-21, but a few first-stage larvae were observed in cold marshes and several pupae in warm roadside pools.

The results, as presented in table 1, show 99 percent less larvae in the treated plots than in the adjacent untreated areas. There were 95 percent less in the outer plot in 1950 than before treatment in

¹ This work was conducted under funds allotted by the Department of Defense to the Bureau of Entomology and Plant Quarantine.

² At time of field work, with the Bureau of Entomology and Plant Quarantine, on leave from Cornell University.

³ At time of field work, captain, on duty on Research and Development Branch, Office of the Quartermaster General, U. S. Army, Washington, D. C.

⁴ At time of field work, lieutenant colonel, from the Georgia Health Department, Atlanta, Ga., on reserve duty with the Preventive Medicine Division of the Office of the Surgeon General, U. S. Army; Washington, D. C.

⁵ At time of field work, lieutenant colonel, from University of Oklahoma Medical School, Oklahoma City, Okla., on reserve duty with the Office of the Surgeon General, U. S. Army, Washington, D. C.

⁶ The writers are indebted to C. N. Husman and military personnel of the Alaskan Command, particularly Captain John Ladda, for their assistance in conducting these studies.

TABLE 1.—Effect of residual DDT from larvicide and adulticide treatments in 1948 and 1949 on mosquito larval populations in 1950 at Eielson Air Force Base, Alaska

Plot	Cumulative DDT per acre (pounds)	Number of dips	Average number of larvae per dip	Percent reduction based on—	
				Untreated areas	1949 population
Outer	0.3 ¹	1,255	0.29	98.7	95
Inner	.7 ²	1,255	.08	99.6	89
Check	0	280	21.9	—	—

¹ 1949 treatments.² 0.3 pound of DDT in 1948 and 0.4 pound in 1949.

1949. The inner plot had shown 86 percent reduction in larvae in 1949, following treatment with 0.3 pound of DDT in 1948. After additional treatments totaling 0.4 pound of DDT in 1949, a further reduction of 89 percent was shown in 1950. The only difference between the two plots was in the average number of larvae per dip, there being over three times as many in the outer plot. Most of the difference was due to a high count in the first transect inside the north boundary. This high count may have been due to poor distribution of the spray, since a low larval kill was also noted in this sector of the plot in 1949. If the dipping records for this transect were omitted in figuring the plot average, the number per dip would be 0.10 instead of 0.29, or about the same as in the inner plot.

The reduction in larval populations in the plots may have been caused in part by reduced oviposition resulting from the control work, but it is thought that the destruction of larvae by the residual DDT was the more important factor. This conclusion was supported to some extent by the fact that larval populations were no greater, as a rule, at the outer boundaries of the plots than in the center, whereas a greater amount of oviposition would be expected there due to the infiltration of adults from adjacent untreated areas.

TESTS OF SEVERAL FORMULATIONS OF DDT AS LARVICIDES. Three DDT formulations used in 1949 (Travis *et al.* 1950) were retested on large plots in 1950. The formulations included an airplane spray solution containing 20 percent of DDT, 20 percent of fuel oil, and 60 percent of

Paccosol; the 20-percent DDT spray with 1 percent of Triton B-1956 (polyglyceride of a glyptal resin) added; and an emulsifiable concentrate containing 20 percent of DDT, 10 percent of Triton X-100 (mono-iso-octyl-phenyl polyethylene glycol ether), and 70 percent of Velsicol AR-50-G. It was planned to compare the different formulations at dosages of 0.05 and 0.03 pound of DDT per acre, but several plots received a lower dosage when openings in the spray boom became plugged and reduced the flow rate by about two-thirds. The dosages used were approximately 0.01, 0.03, and 0.05 pound of DDT per acre, or 25, 75, and 123 ml. of the 20-percent solutions per acre. The test plots were 3/5-mile wide by 2 miles long, and the treatments were applied with a C-47 airplane equipped with underwing booms. Five dips were made before and after treatment in each of 10 marked pools selected in each plot.

The 20-percent DDT spray was applied when the plots were free of ice, but the other treatments were made on a morning when the pools were covered with ice about one-quarter-inch thick. As the ice in these plots did not melt until nearly 12 hours after treatment, dipping was postponed until 48 and 72 hours after application. There was little difference between the two observations, so only the 72-hour data are presented. In addition to more favorable temperature conditions, the six plots receiving the 20-percent spray were treated more satisfactorily than the others, the swaths being more accurately flown and the altitude of flight lower.

As shown in table 2, all of the formu-

TABLE 2.—Mortality of mosquito larvae after 72 hours in plots sprayed from the air with different formulations of DDT

DDT formulation	Dosage per acre		Average number of larvae per dip	Percent control
	Solution (ml.)	DDT (lb.)		
20% solution	78	0.03	33.3	97
	123	.05	14.4	98
20% solution + 1% B-1956	25 ¹	.01	19.1	46
	75 ²	.03	14.4	96
	123	.05	15.3	93
20% emulsifiable concentrate	25	.01	14.9	58
	123	.05	15.3	92

¹ 1 plot.² 2 plots.

lations were highly effective at dosages of 0.03 and 0.05 pound of DDT per acre, larval reductions ranging from 92 to 98 percent. At a dosage of 0.01 pound neither the 20-percent spray with 1 percent of B-1956 nor the emulsifiable concentrate was very effective, but the latter gave slightly higher kills. The results of these tests do not permit a conclusion as to the most effective of the three formulations. They suggest, however, that future evaluation should be made on the basis of somewhat lower dosages than were planned in 1950.

SUMMARY

Observations in large areas treated for mosquito control in Alaska in 1948 and 1949 with accumulative totals of 0.3 and 0.7 pound of DDT per acre showed 99 percent less larvae in 1950 than in adjacent untreated marshes. On the basis of pre-

treatment counts, a plot treated with 0.3 pound of DDT per acre in 1949 showed a larval reduction of 95 percent in 1950. Similarly, a plot treated with 0.3 pound in 1948 had showed a reduction of 86 percent in 1949, and after additional treatment of 0.4 pound per acre in 1949 showed a further reduction in 1950 of 89 percent, or 98.5 percent from the original population in 1948.

Tests with several 20-percent DDT formulations gave 92 to 98 percent control of larvae with dosages of 0.05 pound of DDT per acre, 96 to 97 percent with 0.03, and 46 to 58 percent with 0.01 pound of DDT per acre.

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CHERCHEZ L'HOMME

"While I was working in Nebraska I found a specimen of *Culiseta* that keyed out to *morsitans* on first sight. Later it proved to be something else—the abdominal bands are apical rather than basal and there are other differences. Dr. Harry Pratt and CDC Atlanta checked it and then sent it to Dr. Alan Stone. Dr. Stone believes that it is a new species—on checking the USNM collection he found three more similar specimens from Boston vicinity collected about 1915. All specimens are females and Dr. Stone opposes a description based on females alone. (I agree with him.) I am interested to know if your collection holds any of this species. If I could find a male I would like to co-author on the work."—George A. Thompson, Communicable Disease Center Activities, State Health Dept., Austin 1, Texas.

THE EFFECTIVENESS OF EPN AND SOME OTHER ORGANIC PHOSPHORUS INSECTICIDES AGAINST RESISTANT MOSQUITOES¹

covered 4/166
C. M. GJULLIN, U. S. Dept. Agr., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine, LEWIS W. ISAAK, California Mosquito Control Association, and GORDON F. SMITH, Kern Mosquito Abatement District²

The partial or complete failure of DDT and other chlorinated hydrocarbon insecticides against mosquitoes in California, Florida, and some other states has become a serious handicap to mosquito-control workers in recent years. In California resistance has developed rapidly in the San Joaquin Valley, and in 1952 field applications of larvicides at 10 to 15 times the normal dosages failed to control *Culex tarsalis* Coq. in three mosquito abatement districts and *Aedes nigromaculis* (Lud.) in one district. The high level of resistance found by Gjullin and Peters (1952) in these species and also in *Aedes dorsalis* (Meig.) and *Culex quinquefasciatus* Say suggests that with continued use chlorinated hydrocarbon insecticides will soon be ineffective against all these species in the San Joaquin Valley.

In 1952 the California Bureau of Vector Control, the U. S. Bureau of Entomology and Plant Quarantine, the California Mosquito Control Association, and the Kern Mosquito Abatement District carried on a cooperative project in the Kern District to determine the effectiveness of several organic phosphorus insecticides against resistant mosquitoes. Preliminary tests in the Kern District in the fall of 1951 indicated that EPN might be very effective against resistant mosquitoes. In laboratory tests at Corvallis, Ore., Yates and Lindquist (1952) found it to be the most toxic material they had ever tested against mosquito larvae.

LABORATORY TESTS—The comparative effectiveness of DDT and the three organic phosphorus insecticides is shown in

table 1. On *Aedes nigromaculis* EPN was 67 times as toxic as DDT, while on *Culex tarsalis* it was 171 times as toxic. The LD-50 of EPN on *A. nigromaculis* was 0.000862 p.p.m. Yates and Lindquist (1952) found it to be 0.00135 p.p.m. on non-resistant floodwater *Aedes*. On *C. tarsalis* both tetra-*n*-propyl dithionopyrophosphate and malathion were more effective than DDT, but on *A. nigromaculis* tetra-*n*-propyl dithionopyrophosphate was slightly less effective than DDT.

The toxicity of the three phosphorus insecticides at different temperatures was

TABLE 1.—Parts per million of some organic-phosphorus and DDT larvicides required to give a 24-hour LD-50 of resistant fourth-instar mosquitoes collected in Kern County, Calif.

Insecticide	<i>Aedes nigromaculis</i>	<i>Culex tarsalis</i>
EPN	0.000862	0.000649
Tetra- <i>n</i> -propyl dithionopyrophosphate	.0625	.0178
Malathion	.025	.0185
DDT	.0588	.111

tested on *C. tarsalis* larvae (table 2). EPN was about twice as effective, malathion about four times, and tetra-*n*-propyl dithionopyrophosphate five times as effective at 90° as at 70° F. Yates (1950) found DDT more effective at 63° than at 93° on several species of mosquito larvae, as did Lindquist *et al.* (1946) on adult *Anopheles quadrimaculatus* Say and *Culex quinquefasciatus*.

FIELD TESTS—The tests against *C. tarsalis* larvae were made in areas inundated by the Kern River. The water ranged from a few inches to 2 feet in depth, and usually was partly covered with grass. Most applications on *A. nigromaculis* were made in irrigated pastures in open water

¹ Grafton Campbell, of the Bureau of Vector Control of the California Department of Public Health, assisted in this work.

² The authors wish to thank R. L. Metcalf for valuable suggestions and assistance.

TABLE 2.—Toxicity of organic phosphorus larvicides to fourth instars of *Culex tarsalis* at 70° and 90° F. Average of six replications

Insecticide, p.p.m	Percent Mortality in 24 Hours	
	70° F.	90° F.
EPN		
0.00067	57	91
.0005	46	79
.00025	16	48
.0000125	4	24
Tetra- <i>n</i> -propyl dithionopyrophosphate		
0.033	44	100
.025	17	100
.0167	15	85
Malathion		
0.033	24	85
.025	19	75
.0167	8	40

from 1 to 10 inches deep, but in some plots the water was nearly covered with grass.

The sprays were applied from a jeep with the hose that is standard equipment on these vehicles. The spray head of the hose was equipped with a No. 3 disc, and

the pressure ranged from 150 to 200 pounds per square inch. The diluted larvicides were applied at the rate of 10 gallons per acre in all tests. A few applications were made with a 3-gallon hand sprayer.

One series of tests was made with a Stearman plane, flying 90 miles an hour at a height of 25 to 50 feet. Plane applications were made at the rate of 1.2 gallons per acre.

EPN emulsions were made from a 45 per cent concentrate and EPN suspensions from a 25 per cent wettable powder. The tetra-*n*-propyl dithionopyrophosphate emulsion was made from an 85 per cent concentrate, and the malathion emulsion from a concentrate containing 5.5 pounds of malathion per gallon.

Jeep applications of EPN at 0.035 pound per acre gave kills of 99 and 100 per cent, but higher dosages were required for comparable kills in plane applications (table 3). This was probably due to the smaller volume of more concentrated spray applied by the plane, to the adverse effects

TABLE 3.—Field tests of organic phosphorus larvicides on fourth instars of *Aedes nigromaculis* and *Culex tarsalis*. Average of 2 to 5 replications

Insecticide	<i>A. nigromaculis</i>		<i>C. tarsalis</i>	
	Pounds of Active Ingredient per Acre	Percent Mortality (24 Hr.)	Pounds of Active Ingredient per Acre	Percent Mortality (24 Hr.)
<i>Airplane Applications</i>				
EPN emulsion	0.045	98	0.045	96
	.035	89	.035	89
	.025	45	.025	57
<i>Jeep Applications</i>				
EPN:				
Emulsion	0.035	99	0.035	100
	.025	95	.025	98
	.01	89	.01	97
			.005	57
Suspension	.035	99	.035	100
	.025	98	.025	100
	.01	55	.01	97
			.005	70
Malathion emulsion	.4	99	.3	83
	.3	92	.2	97
	.2	83	.1	67
Tetra- <i>n</i> -propyl dithionopyrophosphate emulsion	.4	93	.3	99
	.3	89	.2	77
	.2	87	.1	76

of light winds in some tests, and to the evaporation of the smaller spray droplets. Malathion and tetra-*n*-propyl dithionopyrophosphate were about one-tenth as effective as EPN.

ROUTINE SPRAYING OPERATIONS—The Kern Mosquito Abatement District began routine application of EPN emulsion at 0.075 pound of EPN per acre with a plane and three jeeps in June after a permit for the experimental use of this insecticide had been granted by the State. Sixteen more jeep operators began using this insecticide in July and during the rest of the season for all control operations except in cities and towns where its use was not covered by State permit. It has given excellent control of larvae in all situations in which it has been applied.

The EPN emulsifiable concentrate is issued in small screw-top bottles, which are carried in compartments of a wooden box in the jeep. One bottle contains sufficient EPN for one tank of diluted emulsion. The operator wears rubber gloves when emptying one of these bottles into the tank, which has been partially filled with water. More water is then added to fill the tank. Instructions for handling the EPN and procedures to be followed in case the skin or clothing is contaminated have been issued to all operators.

TOXICITY HAZARDS—The toxicity of EPN and other phosphorus insecticides both to insects and warm-blooded animals is due to their ability to inhibit cholinesterase activity in the body. In man the cholinesterase activity of the red cells and the plasma of the blood can be used as a measure of this activity. The critical level is considered to be 60 per cent of the normal level for the individual.

The plane pilot and the three jeep operators who began spraying with EPN in the Kern District in June were given cholinesterase-activity tests before they used EPN and at 2-week intervals during the season in order to obtain information on the hazards of these spraying operations. The tests have shown no significant reduction in the cholinesterase level of the men.

Several agencies are investigating the toxicity hazard of EPN to warm-blooded animals. Tests by the Food and Drug Administration and others indicate that EPN has about one-fifth the acute oral toxicity of parathion and that the dermal toxicity of the two materials is about the same. Both malathion and tetra-*n*-propyl dithionopyrophosphate have low acute oral and dermal toxicities in comparison with EPN.

Determination of spray residues on grass has not yet been made, because there is no satisfactory method for recovery of EPN under these conditions. However, no adverse effects have been noted in cattle that have grazed in a pasture sprayed six times at 6- to 10-day intervals with EPN at 0.075 pound per acre.

CONCLUSION—These tests and routine applications indicate that EPN is an effective substitute for chlorinated hydrocarbon insecticides in areas where mosquito larvae have become resistant to these materials. Malathion and tetra-*n*-propyl dithionopyrophosphate are also effective against resistant mosquito larvae. They are less dangerous to handle than EPN, but they may not be so economical to use because of the large dosages required.

SUMMARY

DDT and other chlorinated hydrocarbon larvicides have failed to control *Culex tarsalis* Coq. and *Aedes nigromaculis* (Lud.) in several mosquito abatement districts in the San Joaquin Valley of California.

Laboratory tests were made to compare the effectiveness of three organic phosphorus insecticides and DDT. EPN was found to be the most toxic. The LD-50's of EPN for resistant larvae of *C. tarsalis* and *A. nigromaculis* from the Kern District were 0.000649 p.p.m. and 0.000862, respectively. It is 67 and 171 times as toxic, respectively, as DDT. EPN emulsion was about twice as effective against *C. tarsalis* larvae, and malathion and tetra-*n*-propyl dithionopyrophosphate about five times as effective at 90° as at 70° F.

In field tests in the Kern Mosquito Abatement District EPN killed 99-100 per cent of these mosquitoes when applied from a jeep at 0.035 pound per acre in emulsion or suspension. Malathion and tetra-*n*-propyl dithionopyrophosphate were about one-tenth as effective as EPN.

Routine applications of an emulsion at 0.075 pound of EPN per acre with 19 jeeps and 1 plane in the summer of 1952 were completely effective against these larvae.

Periodic blood tests of four of the men carrying on these spraying operations have shown no significant reduction in the cholinesterase level of their red cells or plasma.

No adverse effect has been noted in cattle that have grazed in one pasture

after six successive applications of this emulsion.

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R SOME TECHNIQUES FOR MOUNTING MOSQUITO EGGS, LARVAE, PUPAE AND ADULTS ON SLIDES

GEORGE J. BURTON, Ph.D.

Entomologist, Engineer Research and Development Laboratories, Fort Belvoir, Virginia

The methods described in this paper have been used by the writer for years in making clear, permanent slides of all stages of mosquitoes.* Good slides do not always result merely by following certain steps. Manual dexterity and care in handling specimens are of utmost importance, and must be acquired. The loss of hairs or scales results in imperfect specimens which may not be identifiable after being mounted.

The mounting media preferred by the writer include (1) xylene-soluble resins such as Clarite, Piccolyte, Permout, or army issue synthetic resin, and (2) alcohol-soluble gums such as euparal and diaphane. These are preferred because there is no yellowing of preparations with

age, and crystallization never occurs. Clarite is colorless, whereas all the other media mentioned have a slight tinge of yellow which is not readily apparent unless the mount is unusually thick. The synthetic resins in the first group are sometimes sold as crystals or nuggets which must be dissolved in xylene to make a mounting medium of desired viscosity. Permout is also available with toluene as the solvent, but xylene is preferred because it does not have the harsh effect that toluene sometimes has on insect tissues. Euparal and diaphane exert a clearing action on the specimen; xylene-soluble media also have some clearing action, as xylene is often used as a clearing agent by itself.

Certain types of equipment are very useful for manipulating specimens, but are not mandatory. The following items have been very useful: minuten nadeln;

* The material presented here in no way constitutes an endorsement by the Department of Defense.

No. 1 insect mounting pins; swab sticks minus cotton swabs; syracuse watch glasses; stender dishes with covers; sharp-pointed tweezers or forceps; a petri dish; fine wire; a small piece of flexible tin, $\frac{3}{4} \times \frac{1}{8}$ inch; medicine droppers, some of which have been cut off at the tip to provide a larger opening; a Bunsen burner or alcohol lamp; fine and medium camel's hair brushes; and a pair of fine-pointed jeweler's pliers. An excellent dissecting and manipulating needle can be made by pushing the point of a safety pin into the flat end of a swab stick, then inserting a minuten or No. 1 insect pin into the puncture with the aid of jeweler's pliers. Five or six such needles are very handy in the laboratory. Several manipulating needles made with No. 3 insect pins are often of value when stronger pins are indicated, as in straightening legs. A short piece of fine wire should be inserted into the end of a swab stick, and the end of the wire looped to $\frac{1}{8}$ or $1/16$ inch; such a loop is very useful in transferring specimens from one container to another. The small piece of tin $\frac{3}{4}$ inch by $\frac{1}{8}$ inch, mentioned above, should be inserted into the split end of a swab stick, and held in place with adhesive tape or scotch tape. The tin should be bent up at the tip, so as to form a J or L. This small spatula is likewise useful in transferring specimens. If desired, sewing needles may also be inserted into swab sticks so as to form manipulating or dissecting needles of various sizes. Such home-made needles can be made at very little cost.

Chemicals required include: 70 per cent ethyl alcohol, 95 per cent ethyl alcohol, xylene, beechwood creosote, and 10 per cent sodium or potassium hydroxide. Absolute ethyl alcohol may be used, if available, but it is not essential. Beechwood creosote may be used over and over again; consequently, a small bottle will last for many months. Clearing solutions of sodium or potassium hydroxide should be changed when considerable amber discoloration is evident. Twenty or twenty-five per cent hydroxide solutions may also be used if quicker action is desired,

although it is recommended that the greater dilutions be used over a longer period of time for best results.

The alcohols are used for extraction of water from eggs, larvae, and pupae. Adults are never placed into alcohol. Beechwood creosote is used for clearing larvae and pupae after dehydration with alcohol. Sodium or potassium hydroxide is used only when beechwood creosote does not clear the specimen satisfactorily. Xylene is used as a premounting dip, and also for removing excess mounting medium as necessary. Ninety-five per cent alcohol is used also for removing excess euparal or diaphane from around the cover glass.

If practicable, eggs, larvae, and pupae should be carried from the field in the water of their natural habitat. Wide-mouth jars are preferred to vials, so as to lessen the possibility of hairs being knocked loose. Judgment should be used concerning the number of specimens per container. Specimens may also be killed in 70 per cent alcohol in the field, but it is preferred that live specimens be brought to the laboratory and killed in hot water. When dropped into hot water just short of boiling, eggs, larvae, and pupae are killed instantly. When they are dropped into alcohol, they twist and turn violently, making it possible for hairs to become detached during their struggles.

A stender dish, 35 x 18 mm. or 50 x 25 mm. is excellent for permanent storage of beechwood creosote used for clearing, since it has a tight-fitting cover which can be easily lifted up for removal. It is recommended that dehydration be carried out also in a stender dish, since a syracuse watch glass does not have sufficient capacity. Larvae and pupae are lifted and transferred with a small wire loop or tin spatula; if a camel's-hair brush is used, hairs may be dislodged. A medicine dropper may also be used for transferring specimens, but there is less chance of hairs or scales being dislodged when a loop or spatula is used. Adults should be lifted by the legs with a pair of tweezers or forceps. After specimens have been

placed on the slide, orientation is accomplished with minuten nadeln. It is essential that adjustment of hairs or parts be done while the specimen is completely covered by the mounting medium, which keeps the hairs in a flexible condition.

Standard histological techniques should be scrupulously followed for cleaning slides and cover glasses, and keeping them free from subsequent contamination with lint, grease, dust and debris.

MOUNTING EGGS. Eggs should be mounted in euparal or diaphane, preferably. They are picked up and transferred with a fine camel's-hair brush or medicine dropper, never with needles. It is not usually necessary for eggs to be cleared; however, egg rafts are often so heavily pigmented that clearing is necessary. Mosquitoes of the genera *Culex*, *Mansonia* (*Taeniorhynchus*), *Uranotaenia*, and *Culiseta* (*Theobaldia*) form egg rafts, but of the latter the subgenus *Culicella* does not. In other genera eggs are laid singly. Often, when a viable egg raft is immersed in 70 per cent alcohol, the unhatched first-instar larvae are forced out of their egg shells for about three-fourths of their length. This results in a mount showing both the egg raft and the mass of first-instar larvae. Clearing of rafts or individual eggs, if necessary, is accomplished in beechwood creosote. Eggs of *Anopheles* and *Orthopodomyia* should be handled very carefully, so as to prevent folding of the lateral membranous flanges of the latter and of the floats of the former. A piece of hollow glass tubing, drawn out very finely, may also be used for transferring individual eggs. Eggs of *Aedes* and *Psorophora* are easily handled, as they have no external membrane. An egg raft may be mounted so as to show either the upper or the lateral aspect. If the egg mass is too large, it may be separated with needles into smaller groups.

MOUNTING LARVAE. Usually only fourth-instar larvae and other skins are mounted, although it may be desired to mount the other instars so as to show changes in size, proportion, and chaetotaxy. Preferably, larvae are killed in hot

water; if they are killed in 70 per cent alcohol, they may fail to clear properly, as in many species of *Aedes* and *Culiseta*. Larvae should be transferred individually, first to 70 per cent alcohol for at least two hours, then to 95 per cent alcohol for at least two hours. Results are best when the dehydrating time is increased up to 24 hours. It has been found that absolute alcohol is not necessary, but may be used if desired. No difference in results has been observed when maximum dehydration has been accomplished with 95 per cent alcohol, as compared with absolute alcohol.

After the initial dehydration, in order to avoid handling of larvae, the writer prefers to remove the 70 per cent alcohol from the stender dish, using a pipette. No hard and fast rule can be laid down about dehydration time, but in general, the larger the larva the longer the time necessary for dehydration. Ninety-five per cent alcohol is poured gently into the stender dish to replace the 70 per cent alcohol removed. After at least two hours, the specimen is lifted up with a wire loop or spatula and transferred to the stender dish containing pure beechwood creosote. The specimen may tend to float for a while, but if it has not sunk below the surface after ten minutes, it should be pushed below the surface of the creosote with a needle or loop. It is not absolutely necessary for the specimen to remain on the bottom, as sufficient clearing will take place even if it remains suspended in the creosote. Some larvae become cleared in ten minutes, whereas others are cleared in one to two hours or more. No definite clearing time can be advocated; frequent examination is necessary. Very often it has been observed that the clearing time varies within the same batch of larvae of a particular species.

When clearing has been completed, the specimen is lifted out of the stender dish, and placed into a syracuse watch glass containing xylene. After one minute, it is removed and placed on a clean slide. The xylene evidently counteracts the effect of minute amounts of water remaining in

the tissues, and is also preparatory for mounting in a xylene-soluble medium. It has been frequently observed that very large larvae, such as those of *Culiseta*, tend to retain minute amounts of water within the tissues, regardless of how many hours dehydration has taken place, even in absolute alcohol. This "bound water" often will cause a whitish precipitate to form around and over the specimen if the one-minute immersion in xylene is omitted. A precipitate seldom appears if this step is followed. Although specimens may be left in beechwood creosote indefinitely, it is recommended that mounts be made as soon as time permits. Occasionally, the beechwood creosote causes the specimen to become brittle, but this does not occur very often.

The mounting medium should not be too viscous, as it will not flow properly when the cover glass is applied. If it is too thin, the medium will seep out, often displacing eggs or larvae toward the periphery of the cover glass. The best viscosity may be determined by trial and error.

A larva must be examined under the stereoscopic microscope prior to being mounted, in order to ascertain whether all hairs are attached. If many specimens of a particular species are available, imperfect specimens may be discarded; however, if a comparatively rare specimen has been found, all such larvae should be mounted, providing the essential characteristics are still apparent. When a rare species is involved, a slightly damaged specimen is better than no specimen at all.

After ascertaining its condition, the larva should be placed upon a clean slide, dorsal aspect up. In culicine larvae, the dorsal aspect is obvious when the respiratory siphon is seen sticking up toward the observer. Any excess xylene remaining around the larva should be removed with any absorbent material. Several drops of mounting medium are then placed directly upon the specimen, so as to cover all the hairs. A sharp razor blade with edge held parallel to the slide, is then placed over the sixth or seventh abdominal seg-

ment, and with gentle pressure the larva is cut through. This procedure will enable the respiratory siphon to flop over on its side, thus bringing into view the pecten teeth, siphonal tufts, anal segment, anal brush, and the comb scales of the eighth abdominal segment. If desired, only a partial cut may be made, thus permitting the posterior segments to remain attached to the anterior portion. In any event, a clear, lateral view of the eighth and ninth abdominal segments is necessary for proper identification.

Anopheles larvae have no respiratory siphon; consequently other means are used to determine whether the specimen has been placed with the dorsal aspect up. Often the palmate hairs are visible without the aid of a microscope; a ten power hand lens will bring these clearly into view. Orientation of *Anopheles* larvae may be accomplished simultaneously with the examination for completeness of the specimen. Since *Anopheles* larvae rotate the head 180 degrees during feeding, they are often killed with the head upside down. An attempt should be made to twist the head back to normal if it is found to be reversed, but this is often difficult to do, and it may be necessary to sever the head from the thorax in order to orient the head properly. Palmate hairs of the right and left sides should be present, but if the specimen is rare it should be mounted regardless of condition until others are obtained.

After the posterior segments have been turned laterally, additional drops of mounting medium should be applied. With the aid of a small glass rod, the medium is then drawn over the slide so as to form a square pattern exactly the size of the square cover glass. This is done for two reasons: to reduce the possibility of the specimen moving while the cover glass is being lowered, and to prevent the formation of bubbles. It is important to have sufficient mounting medium directly upon the specimen, especially if it is a large larva. If the technician is right-handed, he should touch the left edge of the cover glass to

the left edge of the medium, then lower the right edge toward the slide surface with the aid of a needle supporting the right side. If the person is left-handed, the right edge of the cover glass is placed down first, and the corresponding procedure should be followed. There should be little or no movement of the larva, but occasionally, the severed posterior portion becomes disoriented. It can be reoriented as follows: Hold a No. 1 insect mounting pin parallel to the cover glass, carefully insert it under the cover glass from an appropriate direction, gently draw the moved portion into position, then remove the pin. Any specimen may be oriented in this manner. It is important that the pin be perfectly parallel to the slide, otherwise the cover glass will be pushed off. No ringing of the cover glass is necessary. The slide should be left in a horizontal position in a drawer or slide box for several days to a week, depending on the thickness of the mount. Usually, the medium begins to harden within twenty-four hours; when Clarite is used, the setting time is usually much shorter. Larval skins are also mounted as described above.

Only by experience will the person making slides learn just how much mounting medium is necessary for any particular specimen. The length, breadth, and height of the specimen will assist in approximating the amount which is necessary. If too much has been added, the excess will seep out quickly at the edges of the cover glass. If this happens, the slide should be kept perfectly horizontal, and, as seepage occurs, the excess should be wiped away with a small piece of paper towelling or a small square of cloth. When the seepage ceases wipe away traces of the medium at the right and left edges, using a cloth dipped in xylene. Never clean away any excess from around the top and bottom edges, as there is usually very little free space between the cover glass and edge of the slide, thus making it easy to move the cover glass and spoil the mount if care is not taken. When using diaphane or euparal, excess medium

should be removed with a cloth dipped into 95 per cent alcohol, never 70 per cent alcohol. If a round cover glass is used instead of a square one, the same procedures are to be followed, except that the mounting medium should be spread to the size and shape of the cover glass prior to lowering the latter.

Often the amount of mounting medium is underestimated, but the remedy is simple. The slide is kept in a horizontal position, and slight pressure is placed upon the cover glass with the tip of a needle until all the existing medium moves to the lower half of the mount. Using a thin glass rod, additional medium is applied beneath the top edge of the cover glass, one drop at a time, until the empty space becomes filled with the medium. The slide may be tilted very slightly, if necessary, to assist the newly-added medium in flowing down to mingle with the medium already on the slide. A slight excess should be added to compensate for evaporation. The additional medium may also be added with a medicine dropper having a narrow opening, but extreme care should be used to avoid bubbles, and to avoid dropping the medium on top of the cover glass.

The removal of an air bubble depends on its location. A bubble located near the edge of the cover glass may be removed by inserting a needle beneath the cover glass and working the bubble toward the edge. When it reaches the edge, touch it with the point of a twisted piece of paper towelling, or small cloth; the bubble will usually be absorbed. If the air bubble is located too far in from the edge, and is in the upper half of the mount, tilt the slide slightly by holding the lower edge of the slide to the table and raising the upper edge. The bubble will then travel toward the upper edge of the cover glass, from whence it may be removed with the point of a twisted paper towel. If the bubble is in the lower half of the mount, the opposite procedure is followed; i.e., the lower edge of the slide is raised, and the bubble will flow gradually toward the lower edge. In other words, a bubble tends to work

its way toward the edge which is raised.

Another convenient method of removing an air bubble is to use a very fine pipette made by drawing out a piece of glass tubing. Insert it under the edge of the cover glass and aspirate the bubble out. The bulb should be squeezed *before* inserting the pipette underneath the cover glass, otherwise bubbles will be added instead of removed. Should there be too many bubbles, or too large a bubble, it is recommended that the cover glass be removed, the bubbles wiped away, and a new cover glass added.

Very often, when a slide is examined the day after being prepared, evaporation at the edges or corners may be observed. Additional mounting medium should be added with a medicine dropper or narrow glass rod. It is a good idea to add a little of the medium to excess. Once the medium has hardened, it is not feasible to attempt to remove any excess from around the cover glass, as it may be covered over when slide labels are affixed. If removal of hardened medium is absolutely desired, it may often be accomplished by scraping carefully with a single-edged razor blade, taking care not to touch the cover glass.

There should be no halo around the specimen. When mounted, the larva should present a clear outline, just as if it were alive and being examined in water. Should a whitish halo appear after hardening of the medium, the cover glass should be removed, the larva should be replaced into xylene for one minute, and then removed. Should the halo persist after the larva has been mounted for the second time, it is evident that incomplete dehydration has taken place, consequently, the larva should be either replaced into 95 per cent alcohol, or else discarded if many such specimens of the same species are on hand.

If desired, the larva may be mounted in euparal or diaphane from 95 per cent alcohol, with or without clearing in beechwood creosote, as the alcoholic mounting media act as clearing agents themselves to some extent. Such a mount will, how-

ever, take much longer to dry than when a xylene-soluble medium is used.

The specimen should be mounted directly in the center of the slide. The writer prefers to use two labels. The label to the left of the cover glass should have printed upon it the complete scientific name of the specimen, the date collected, the locality where collected, the type of water accumulation from which collection was made, and the name of the collector. The label at the right should have printed upon it the chief characteristics used in identifying the specimen to the particular species, so that any person can readily check the identifying characteristics against the specimen itself. This procedure is valuable in teaching taxonomy, and avoids constant reference to keys; it is also useful when a field worker sends a mounted specimen to his headquarters located far away. It is entirely possible that the field entomologist may have keys, whereas his headquarters may lack them. If the label at the right is included, the receiving entomologist will have all information available on the slide mount. If the field entomologist had sent the specimen with only the determined genus and species, and the receiving entomologist had no key for determination of the specimen, the receiving entomologist might not know which features of the larva were diagnostic for the particular species.

MOUNTING PUPAE. The pupal characteristics of value in identification are the size and shape of the respiratory trumpets, the shape of the cephalothorax, and the chaetotaxy of the abdominal segments and paddles. Ordinarily one does not attempt to determine the species of a particular pupa, for the adult will emerge soon and lend itself more readily to identification.

A pupa is ordinarily too bulky to be mounted on a flat slide without being modified first, but such a mount is possible with slight distortion. After being dehydrated in 95 per cent alcohol, a pupa is placed between two slides in a petri dish containing 95 per cent alcohol. Using slight but continuous pressure upon the

upper slide directly over the pupa, the latter may be flattened slightly without being crushed. The abdomen may be shown either in its natural lateral aspect, or may be twisted around so as to show the dorsal chaetotaxy. The specimen is then placed into beechwood creosote for clearing, and later mounted as described for larvae. The mount may tend to be high, but pressure on the cover glass will usually produce an acceptable mount. More mounting medium is required in mounting pupae than in mounting larvae, due to the height of the pupa. It is better to add excess medium and then wipe the excess away, than to underestimate and attempt to add medium later. As the pupa becomes enveloped by the mounting medium, it will tend to flatten. Evaporation of some of the medium often takes place by the next day, due to the height of the mount, consequently additional medium should be applied as evaporation occurs. Complete drying of such a high mount requires several weeks to a month with all media except Clarite, the latter usually drying in one to several days.

If desired, a pupal skin may be mounted instead of the entire pupa, after emergence of the adult. Clearing is not necessary in this case, as the skins are quite transparent. The dorsal chaetotaxy may be made apparent by detaching the cephalothoracic portion, or by twisting the abdomen while the cephalothoracic portion is attached. The skin may be mounted in euparal or diaphane directly from 95 per cent alcohol, or in a xylene-soluble medium directly from xylene. Labels should be affixed as described under mounting of larvae.

MOUNTING ADULTS. Mounting of adults on slides should be a supplement to, not a substitute for, pin mounts. The writer developed this method as a means of preserving permanently certain anatomical features of mosquitoes, especially for use in teaching. An entire male or female mosquito may be mounted on a flat slide, making the following features easy to study: wing scale patterns, ring patterns on legs, color patterns of tergites and

sternites (to some extent), mouth parts, antennae, eyes, head tufts, and lateral thoracic bristle groups. White stripes, white bands, and other whitish or yellowish markings usually retain their color, but in the genus *Aedes* the white markings become cleared. It has been found that white markings of *Culiseta* (*Theobaldia*) and *Anopheles* show up very well.

Only reared, unfed adults are mounted, as they possess all the bristles characteristic of the species, are quite transparent, and have a minimum of water in the body. Following emergence from the pupal case, the adult is killed with ether or chloroform. As soon as it is dead, it is placed on its side on a clean slide, and synthetic resin mounting medium is dropped upon it, thus gluing it down. Additional drops are applied upon the head, legs, and abdomen, but most of the medium is concentrated upon the heaviest parts of the body. The wing nearest the technician will give the greatest trouble, as it tends to fall so as to obscure the thorax and abdomen. The opposite wing will give less trouble, as it can be glued down readily with the mounting medium. Fracturing the axillary sclerites at the wing base often tends to permit manipulation of the wings as desired. Pins should not be pressed against the wing membrane, as it will tear. Insert the pin next to the costa for easiest manipulation of the wing. The mount may not be very high, as the adult has not been given the opportunity to feed. Only a square cover glass should be used, and should be lowered in the manner already described. Should the wings, legs, antennae, or mouth parts tend to move out of position after the cover glass is applied, they may be returned to their former position by using a No. 1 insect pin carefully inserted beneath the cover glass.

It is usually unnecessary to dissect out the individual mouth parts, as they tend to separate away from the labium of their own accord when the mounting medium is dropped upon the proboscis. Should separation not be complete, it should be accomplished with minuten nadeln under

a stereoscopic microscope. The labrum-epipharynx (U-shaped in cross section), mandibles (sharp-pointed), maxillae (distally finely serrated), hypopharynx (stylet-like) and labium (sheath-like, terminating in a pair of labella) should be discerned after the mount has been made. When making mounts of male mosquitoes, the mouth parts may or may not separate out. Details of the antennae and palps are readily discernible in all mosquitoes if the mounts are prepared properly. Mounting of an adult on a slide may take about 15 minutes or more, but the results are worth the effort. Dorsal and ventral views are best seen in mosquitoes mounted on pins. It is to be re-emphasized that slide mounts are supplements to, not substitutes for pinned mosquitoes.

Any part of the mosquito body may be mounted separately. Heads, legs, and wings may be dissected from the body and mounted alone. Wings must never be put into liquids. To mount a pair of wings, remove them carefully from the thorax, orient them on a clean slide, spread a few drops of a xylene-soluble medium over the wings, and apply a cover glass. Should the wings move out of position, insert a No. 1 insect pin beneath the cover glass, and carefully move them back into position. If mosquito heads or legs are to be mounted individually, they should be separated from the body *prior* to clearing in beechwood creosote, as it is more difficult to perform the dissection after clearing.

To mount male genitalia, snip off the tip of the abdomen prior to dehydration, clear in beechwood creosote, and mount after drawing out the parts with fine needles. It is often advantageous to clear with hot or cold 10 per cent sodium or potassium hydroxide instead of beechwood creosote, for better depigmentation. The clearing time may be shortened considerably when the hydroxide is warm or hot. The freshly-clipped genitalia should be placed into the hydroxide solution, and after clearing has progressed to the desired point, the genitalia should be placed in tap water or distilled water for one or two

hours. Dehydrate with 70 per cent alcohol for two hours, follow by 95 per cent alcohol for two hours, and then mount in euparal or diaphane; otherwise the genitalia may be transferred to xylene for one minute following 95 per cent alcohol, and mounted in one of the synthetic resins. Drawing out of the parts of the genitalia may be easier to accomplish after washing in water, instead of after dehydration. The hardening action of the alcohol may tend to keep the parts in place.

SUMMARY. Some successful techniques for mounting mosquito eggs, larvae, pupae, and adults on slides have been presented. Briefly, the steps are as follows:

Mounting Eggs. (1) Kill in hot water (preferably) or 70 per cent alcohol. (2) Dehydrate in 70 per cent alcohol for two hours. (3) Dehydrate in 95 per cent alcohol for two hours. (4) Mount in euparal or diaphane.

Mounting Larvae. (1) Kill in hot water (preferably) or 70 per cent alcohol. (2) Dehydrate in 70 per cent alcohol for 2-24 hours. (3) Dehydrate in 95 per cent alcohol for 2-24 hours. (4) Clear in beechwood creosote for at least 10 minutes. Remove when clearing is complete. Store indefinitely in beechwood creosote, if desired. (5) Place in xylene for 1 minute. (6) Mount in a xylene-soluble medium, such as synthetic resin, Piccolyte, Clarite, or Permunt; *or*, mount in euparal or diaphane, omitting step (5).

Mounting Pupae. (1), (2), (3)—same as for larvae. (4) Place pupa between 2 slides in petri dish filled with 95 per cent alcohol. Apply slight pressure so as to flatten pupa slightly. (5) Follow steps (4), (5), and (6) under *Mounting Larvae*.

Mounting Adults. (1) Kill emerged, unfed adult with ether or chloroform. (2) Place on slide as desired, cover with synthetic resin mounting medium or equivalent. (3) Orient wings, legs, head, and mouth parts, and apply cover glass.

Continued practice is necessary for achieving success in mounting. The technician will often develop variations in technique or new techniques after con-

siderable experience. Manual dexterity is necessary for orientation of parts of the specimen which are comparatively fragile. Various mounting media other than those mentioned herein have been used by entomologists, and may be tested by the

newcomer to slide-making. The methods presented here have been time-tested, and, if followed faithfully, should result in permanent slides of mosquito eggs, larvae, pupae, and adults, of which the entomologist can be proud.

A TECHNIQUE FOR MICROMANIPULATION OF MOSQUITOES¹

ROBERT CHARLES WALLIS²

The importance of insects in basic research has become more generally recognized in recent years. Many possible lines of investigation have been pointed out in physiological research by Roeder (1952). In one of these, using the mosquito as an experimental animal, Roth (1952) describes physiological experiments requiring microsurgery of the adult mosquito. Shambaugh (1952) points out the importance of microinjection techniques for studies in toxicology and physiology, and describes a microinjection technique for *Aedes aegypti*. However, in studies involving mosquito microsurgery and microinjection, extreme difficulty is encountered in the handling of the mosquito due to its very fragile structure and small size. Physical damage of mechanically holding the mosquito, surgical shock, heat desiccation, and prolonged anesthesia in such operations all tend to produce a high operative mortality rate. Studies in this laboratory requiring micromanipulation of living mosquitoes for microsurgery have resulted in an improved, simplified technique that has effectively lowered post-operative mortality, and reduced preparation time for experiments that require operations on large numbers of mosquitoes.

This success is due principally to the introduction of a suction pipette as a holding device to replace pinching or other friction holding instruments for securing the anesthetized mosquito while operating under a dissecting microscope. The suction pipette, pictured in Fig. 1, was made by drawing out a quarter-inch glass tube to a small oval tip sized 1 by 2 millimeters. This tip was then covered with grid by cementing on a piece of 51 gauge nylon cloth material. The pipette was attached with rubber tubing to a water pump suction device, and sufficient suction maintained to securely hold an adult mosquito to the grid tip of the pipette.

In use, the adult mosquitoes were exposed briefly to CO₂ gas in small cartons, and when anesthetized, the rubber tubing to the pipette was pinched to shut off the suction, the tip of the pipette placed close to the scutum of the mosquito, the tubing released to start suction in the pipette so that it firmly secured the mosquito against the grid tip, as shown in Figure 1. In this manner the mosquito is held in place by gentle pressure against the relatively strong thick integument of the scutum, with little damage to the mosquito. Also, as shown in Figure 1, there is a minimum surface area occluded by the holding device, and the legs and other appendages are left freely accessible. Holding a mosquito thus, it could be picked up and manipulated freely under the field of the dissecting microscope without physical damage. Also, held securely in this man-

¹ Study aided by grant from National Microbiological Institute, The U. S. Public Health Service, Bethesda, Maryland.

² Department of Parasitology, Sch. of Hyg. & Pub. Hlth., The Johns Hopkins University, 615 N. Wolfe Street, Baltimore, Md.



FIG. 1. Female *Aedes polynesiensis* held by suction to the tip of a nylon covered pipette for manipulation under a dissecting microscope.

ner it was not necessary to maintain full deep anesthesia and operative mortality due to overanesthetization was avoided.

The operative field under a dissecting

microscope was covered with a transparent plastic hood. Within this hood cool moist conditions were maintained to protect the mosquito from the effects of heat desiccation. Heating and drying were also reduced by supplying a light source from outside the plastic hood. Instruments were standard for such techniques, including fine glass rod points and hooks, iris scissors, and small scalpels made from bits of razor blades (Roth, 1952).

By using the suction grid tip pipette method of holding mosquitoes for microsurgery, operation time, operative and post-operative mortality have been greatly reduced. Such operations, in numbers, as single and multiple leg amputations, tarsiectomies, palpectomies, and antennectomies have been performed on *Aedes aegypti*, *A. polynesiensis*, *Anopheles quadrimaculatus*, *An. freeborni*, *Culex pipiens*, and *C. fatigans*. It is believed that the use of the suction pipette may be of use in the micromanipulation of mosquitoes for other operations as well as for the handling of other delicate insects.

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Are your name, address and status with respect to your membership correctly given in the lists? See pages 38-54. Please notify the Secretary of any errors.

OBSERVATIONS OF EARLY SPRING ACTIVITY OF *CULISETA INORNATA* (WILLISTON) (DIPTERA, CULICIDAE) IN SOUTH CENTRAL NEBRASKA¹

GEORGE A. THOMPSON, JR.

S. A. Sanitarian (R) C. D. C. Activities, Texas State Department of Health, Austin, Texas

The activity of *Culiseta inornata* (Will.) was observed during February, March, and April, 1950 at a borrow pit located about 3 miles south of Kearney, Nebraska. The observations confirm the overwintering of this species in the adult stage.

As early as February 8, water appeared in small holes in the surface of the ice. In midafternoon the air temperature was 40° F. and that of the water was 34°. Some Collembola and copepods were active; no other aquatic life was noted. On February 20, water was present between the surface ice and the bottom ice. The surface ice was softening and becoming porous. Collembola and copepods still were the only active life found. On March 6, the second of two consecutive days when the air temperature reached 71°, the water temperature was 58°. No stages of mosquitoes were found although small numbers of other aquatic insects were active.

No further observations were made until March 31, at which time no larvae, but many egg rafts were found. The majority of the eggs were fully pigmented. Samples of the incompletely pigmented eggs that were held at room temperature became completely pigmented within 12 hours. Dissection of some of the fully pigmented eggs revealed, at 50X magnification, no embryonic development. Several of the darker egg masses that were kept at room temperature produced larvae on April 3.

On April 4, following a heavy snow, the water temperature was 38° under a 5-inch blanket of snow. No larvae were

found at this time although a thorough search was made. First instar larvae were found on April 11. Some larvae appeared to be at least halfway through the first instar. Adult mosquitoes were conspicuous, flying about the borrow pit as the vegetation was disturbed. A good sample of the population was obtained by sweeping; all were *C. inornata*. Larvae were reared until positive identification could be made. These were also all *C. inornata*.

During the time that the borrow pit was under observation, several other ponds were checked to determine developments in the vicinity of the borrow pit. All other ponds were several days later developing aquatic life than was the borrow pit. This probably was due to the pit being so located that it received the full benefit of the warmth from the sun but was protected from the northwest winds.

Eggs were found shortly after the first dates that the minimum temperature remained above 32° and after there was sufficient ice-free water for oviposition. Diligent search failed to reveal any stages prior to the finding of eggs. The presence of eggs that were not fully pigmented indicated recent oviposition. The occurrence of adults at about the time of finding the eggs, confirms the probability of recent oviposition. The author feels that it would have been impossible for larvae or pupae to have existed in the borrow pit prior to the finding of eggs, and to have escaped observation. The checking of other ponds eliminated the possibility of there being more advanced breeding in them.

These observations indicate that the *C. inornata* under consideration could have overwintered only as adult mosquitoes.

¹ From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Georgia.

PICTORIAL KEYS TO THE MOSQUITOES OF MEDICAL IMPORTANCE

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The rapid identification and separation of the mosquito species of primary medical importance is frequently a difficult problem for the field mosquito-control worker, particularly in parts of the world where literature on the subject is not available. To assist health workers in various parts of the world to meet this problem, the Department of the Army, through a transfer of funds to the Bureau of Entomology and Plant Quarantine, is developing a series of *pictorial keys* to the mosquitoes of primary medical importance. The keys indicate both the principal malaria vectors of the area and those species that are known or suspected of transmitting other diseases. The accompanying key is the first of this series.

It is hoped that these keys will serve as a rapid and convenient tool for anyone whose work requires identification of the principal species of medical importance in the area concerned, whether he be a professional taxonomist or a mosquito-control worker.

The keys are so constructed that they not only separate those species of primary medical importance for the given area, but also eliminate from consideration all other species known to occur there. However, there are serious gaps in knowledge of the mosquito fauna of many areas. Suggestions and comments will therefore be welcomed, especially from those who have an opportunity to use the keys and those with special information on the faunas of the countries.

I. KOREA

KATHIRYN M. SOMMERMAN

United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine

Of the six species of *Anopheles* definitely recorded from Korea, only one is of primary medical importance. This is *Anopheles sinensis*, one of the most commonly encountered mosquitoes in Korea and an extremely efficient vector of malaria throughout that country. It is also an important intermediate host of *Wuchereria bancrofti* and has been suggested as a possible vector of Japanese B encephalitis. Characteristically, though not always, it breeds in and near rice paddies and smaller ground pools, being an open-country, fresh-water breeder. *Anopheles pattoni* and *Anopheles labranchiae atroparvus*, the latter a member of the *maculipennis* complex, have been included because their ranges may extend into this country from nearby areas where

they are important vectors of malaria. The former is a brackish or fresh-water breeder, and the latter inhabits rain pools and small streams. According to available reports, *Anopheles lindesayi japonicus*, a high-altitude breeder, is a very poor vector. The remaining species of *Anopheles*, not included in this key because of their lack of importance, are *edwardsi*, *koreicus*, *pullus* (of uncertain validity) and *sineroides*.

Thirty-five species of non-anopheline mosquitoes have been recorded from Korea. Although experimental evidence has incriminated many of them in the transmission of Japanese B encephalitis, *Culex tritaeniorhynchus* is the only one so far found to be infected in nature, and it is

MOSQUITOES OF MEDICAL IMPORTANCE — KOREA

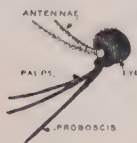
FEMALES

IMPORTANT
A SPECIMEN MUST HAVE
ALL CHARACTERS LISTED
FOR THAT SPECIES

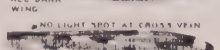
[MALES HAVE BUSHY ANTENNAE () AND DO NOT BIT!]

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UNDER FUNDS ALLOCATED BY
SECRETARY OF ARMY
MAY 1952

LONG PALPS ON
ANOPHELES

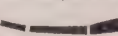


[THIS SPECIES IS NOT KNOWN FROM KOREA BUT MIGHT OCCUR HERE] = *ANOPHELES ATROPARVUS*

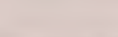
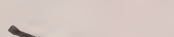


PATTERN ON HIND TARSUS

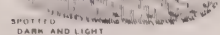
PATTERN ON PALP



= *ANOPHELES SINENSIS*



= *ANOPHELES PATTONI*



SHORT PALPS ON
ALL OTHERS

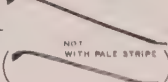


PATTERN ON THORAX

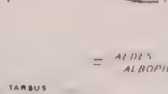
PROBOSCIS

WING

MID TIBIA



= *CULEX TRITANIORHYNCHUS*



= *AEDIS ALBOPICTUS*

NO SIPHON ON *ANOPHELES*



INNER CLYPEAL HAIR FAR APART

= *ANOPHELES PATTONI*



INNER CLYPEAL 30-BRANCHED, CLOSE, AT LEAST

= *ANOPHELES ATROPARVUS*

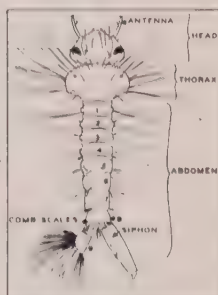


ABDOMINAL PLATE B

NARROWER THAN SPACE BETWEEN PALMATE HAIRS

= *ANOPHELES SINENSIS*

WIDER THAN SPACE BETWEEN PALMATE HAIRS



SIPHON ON ALL OTHERS



SIPHON

ANTENNA

COMB SCALE

BODY HAIR



= *AEDIS ALBOPICTUS*



= *CULEX TRITANIORHYNCHUS*



= *CULEX TRITANIORHYNCHUS*

suspected of being the chief vector of that disease. It is principally a night biter, and its biting and breeding habits correspond well with the epidemiology of Japanese B encephalitis.

Aedes albopictus is commonly encountered in Korea, and is regarded as the only vector of dengue fever there. Like

Aedes aegypti, which apparently does not occur in that country, it breeds in artificial containers near human habitations and is almost completely domestic. Dengue is not at present a common disease in Korea, but if introduced from nearby areas the presence of its vector might cause it to become important again.

II. FORMOSA

KATHRYN M. SOMMERMAN AND RICHARD H. FOOTE

United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine

Although 20 anophelines are known to occur in Formosa, only the 6 species treated in the keys are of primary medical importance because of their ability to transmit malaria. *Anopheles sinensis* is believed to be the principal vector in the plains throughout Formosa. It is the most prevalent anopheline and is associated in large part with rice culture in that country. Particularly in foothill regions, *Anopheles minimus* is known to be an important vector. Breeding in moving fresh water, it occurs in large numbers and is easily collected in houses and cattle sheds. *Anopheles fluviatilis*, the larvae of which cannot be separated from those of *minimus*, is not definitely known to occur in Formosa. Like *minimus*, *Anopheles maculatus* is a foothill stream breeder. In Malaya and other areas it is associated with an intense malaria, but in Formosa this species is quite rare and is included in the keys only because of its potentially great ability to transmit malaria. Three species of *Anopheles*—*tesellatus*, *annularis* and *sundaicus*—have been incriminated in malaria transmission

only at certain times of the year and in restricted localities in Formosa. The first two typically breed in pools formed in sugar cane fields near the end of the rainy season and may reach high densities at that time. *A. sundaicus* breeds in direct sunlight in small pools without vegetation, such as those formed during the drying of the larger streams. It has been incriminated in the past in certain outbreaks of malaria in Formosa.

The remaining *Anopheles*, known or assumed to be present in Formosa, are *aikeni bengalensis*, *barbirostris barbirostris*, *barbumbrosus*, *gigas baileyi*, *insulaeflorum*, *jeyporiensis candidiensis*, *kochi*, *leucosphyrus*, *lindesayi*, *ludlowi*, *splendidus*, *subpictus indefinitus*, and *vagus*.

Of the approximately 60 nonanopheline mosquitoes, only 2 are of outstanding medical importance. These are *Aedes aegypti* and *Aedes albopictus*, both of which transmit dengue fever. Both are urban breeders, utilizing all kinds of artificial containers near human habitations.

MOSQUITOES OF MEDICAL IMPORTANCE — FORMOSA

IMPORTANT
A SPEC MEN MUST HAVE
ALL CHARACTERS LISTED
FOR THIN SPECIES

MALES HAVE BUSHY ANTENNAE (Hatched) AND DO NOT BITE

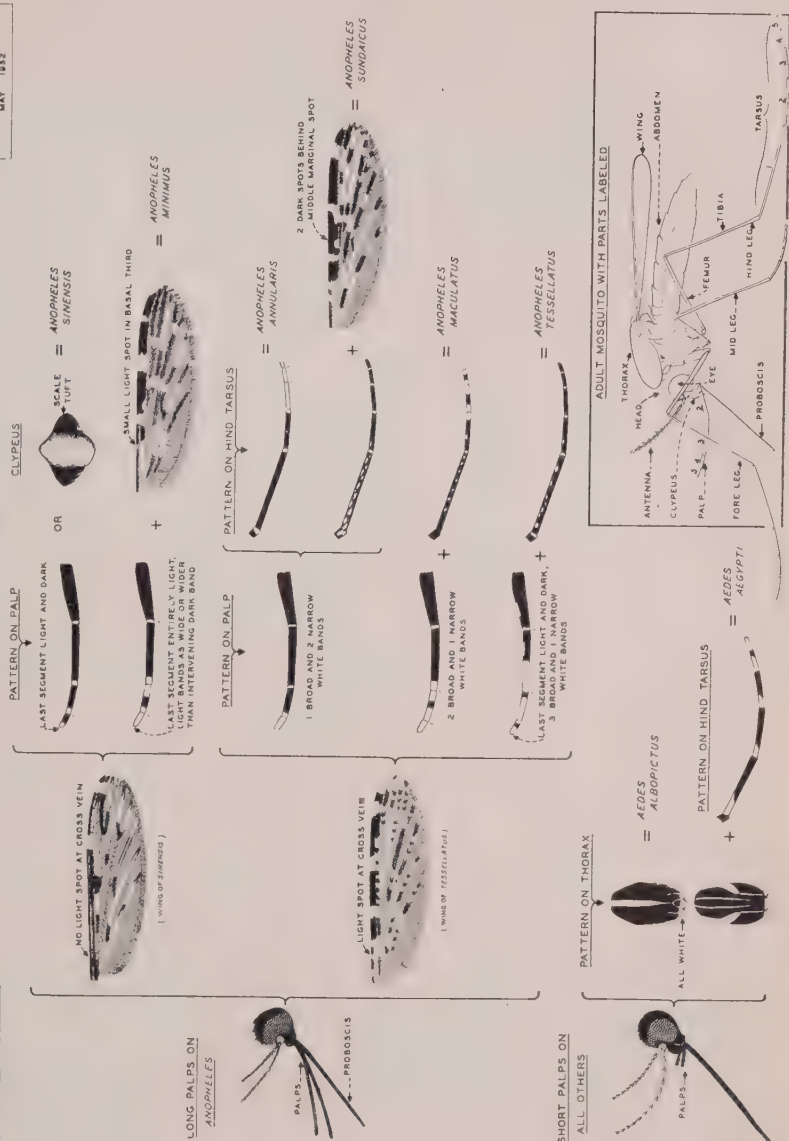
MALES HAVE BUSHY ANTENNAE (Hatched) AND DO NOT BITE

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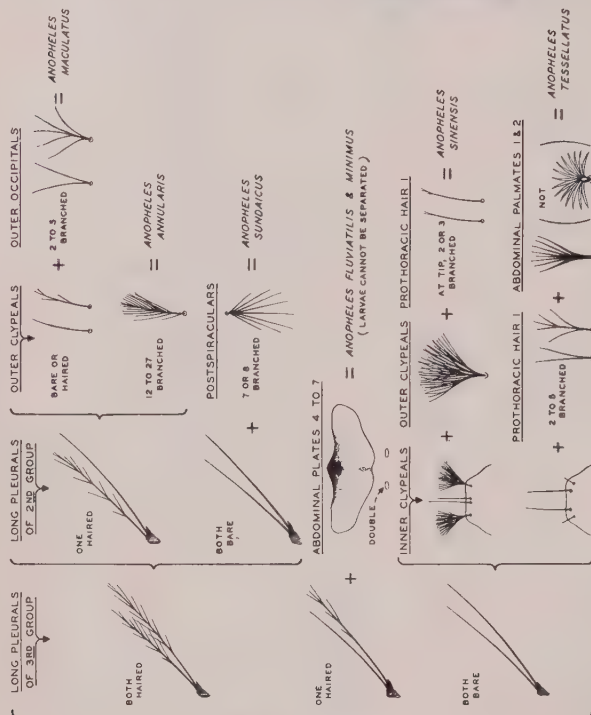
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UNDER FUNDS ALLOTTED BY
SECURITY MAT.
MAY 1952



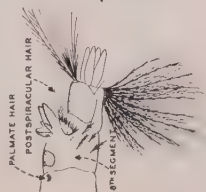
IMPORTANT
A SPECIES OF MOSQUITO MUST HAVE
ALL CHARACTERS LISTED
FOR THAT SPECIES

MOSQUITOES OF MEDICAL IMPORTANCE — FORMOSA

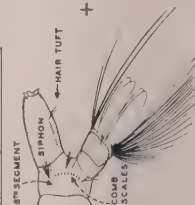
FULL-GROWN LARVAE



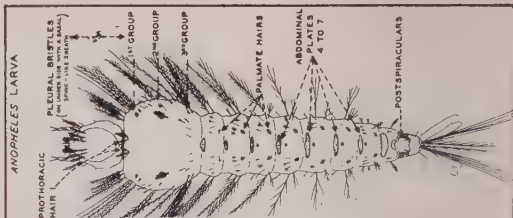
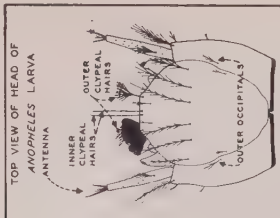
NO SIPHON ON ANOPHELES



SIPHON ON ALL OTHERS



INSECT IDENT. BEP. USA
USDA
SECRETARY OF AGRICULTURE
MAY 1952



ASSOCIATION NEWS

PERRY W. RUTH

Perry W. Ruth who died in Norfolk, Virginia, on December 9, 1952, at the age of seventy-six, was a man of restless energy who directed his enthusiasm into many channels during his long life. He was a native of Maryland and came to Norfolk about the turn of the century. Ever since, until the time of his death, he made himself an important part of the community's life.

He was an active churchman, having organized a Sunday school class of young men many years ago, which is still known as the Perry W. Ruth Young Men's Bible Class. He was a member of several civic organizations and was the oldest ex-president of the Norfolk Builders and Contractors Exchange. Mr. Ruth was widely known for his activities in connection with baseball, dating back to 1911; and at one time he was general manager and part owner of the Norfolk Tars. For a num-

ber of years, he headed a construction company that bore his name. He was in the butter and egg business as well as several other enterprises.

In 1933, he entered the field of mosquito control at an age when many men are beginning to let up. He headed the control work in Norfolk City and, in particular, created a public awareness of the problem. In 1946, he was president of the American Mosquito Control Association; and in 1947, he was the first president of the Virginia Mosquito Control Association. He will be remembered in mosquito control work for his role in the fight against mosquitoes and especially for his enthusiasm in public relations.

He will be greatly missed not only by his many friends in his adopted city but, also, by his many mosquito control friends across the continent.

R. E. DORER

REPORT OF THE AMCA GOOD NEIGHBOR CLUB

FEBRUARY 15, 1952 to FEBRUARY 15, 1953

A total of \$353.00 has been received by the Good Neighbor Club during the past year. An unusually fine contribution of the New Jersey Mosquito Extermination Association Proceedings, bound, and a set of the Journal of the National Malaria Society were received from Prof. Willem Rudolfs of the New Jersey Agricultural Experiment Station. Twenty-six copies of the "Mosquitoes of New Jersey" were received from the Middlesex County Mosquito Extermination Commission. These splendid donations are gratefully acknowledged by the Club and our best efforts will be used in placing them in

foreign hands where they will be of greatest usefulness. All of the cash (\$353.00) received this year will be used to purchase copies of Bulletin No. 3 on the artificial rearing of mosquitoes for laboratory use. The Association hopes to publish this bulletin within the next few weeks. Copies of the bulletin so purchased will be sent to foreign entomologists engaged in mosquito control and research. To date a total of \$817.42, as well as a great number of valuable books and periodicals, has been received by the Club during the past six years. All of the cash heretofore has been used to pay for subscriptions to

MOSQUITO NEWS for shipment to foreign libraries. The following friends and members have contributed to the Club this year:

Helen Sollers, Washington, D. C.; Helen Louise Trembley, Bethesda, Md.; Dinniemaude Jensen, Bethesda, Md.; C. R. Twinn, Ottawa, Canada; R. E. Dorer, Norfolk, Va.; D. L. Collins, Albany, N. Y.; A. W. Lindquist, Corvallis, Ore.; T. D. Mulhern, Fresno, Cal.; T. G. Raley, Selma, Cal.; D. M. Rees, Salt Lake City, Utah; E. G. Batte, Haddonfield, N. J.; F. C. Bishopp, Washington, D. C.; H. H. Stage, Washington, D. C.

Concession Supply Company, 3916 Secor Road, Toledo 13, O. (Mosquito light-traps); Dyna-Fog Corporation, Dayton Municipal Airport, Vandalia, O. (Dyna-Fog applicators); D. B. Smith and Company, Utica 2, N. Y. (Sprayers and spray pumps); Gulf Research and Development Company, P. O. Drawer 2038, Pittsburgh 30, Pa. (Quick action Gulf-spray, Gulf-spray aerosol bombs, Gulf livestock spray and Gulf Trak moth spray—6 per cent DDT); American Cyanamid Company, 30 Rockefeller Plaza, New York 20, N. Y. (Agricultural chemicals); Glyco Products Company, 26 Court Street, Brooklyn, N. Y. (Chemicals and non-ionic emulsifying agents); H. D. Hudson Manufacturing Company, 589 East Illinois Street, Chicago 11, Ill. (Sprayers and dusters); Atlas Powder Company, Wilmington 99, Del. (Chemicals and explosives); The Spartan Company, 2900 Emerson Avenue, South, Minneapolis, Minn.; Lawrence Aero Mist Sprayer Corporation, 3 Bank Row, Greenfield, Mass. (Mist sprayers); Seacoast Laboratories, Inc., 156-158 Perry Street, New York 14, N. Y. (Pyrethrum insecticides); Insect Wire Screening Bureau, 74 Trinity Place, New York 6, N. Y.

Sentco, Inc., 2400 Tamarind Avenue, West Palm Beach, Fla. (Tossits—water-soluble capsules containing insecticides); Bridgeport Brass Company, Bridgeport 2, Conn. (Insecticidal aerosols); Pennsalt International Corporation, Widener Building, Philadelphia 7, Pa. (DDT, BHC, toxaphene, chlordane, aldrin, and other

insecticides); Stauffer Chemical Company, 1496 East Fremont Road, Mountain View, Cal. (DDT, lindane, BHC, toxaphene, parathion, chlordane, aldrin, and dieldrin insecticides); California Spray-Chemical Corporation, Richmond, Cal. (Isotox dusts and sprays containing lindane, toxaphene, and DDT for controlling mosquitoes); Geigy Company, Inc., 89 Barclay Street, New York 8, N. Y. (Originators of DDT insecticides); Velsicol Corporation, 330 East Grand Avenue, Chicago 11, Ill. (Chlordane insecticides, solvents, and herbicides); Refined Products Corporation, Lyndhurst, N. J. (Emulsifying agents for use in insecticides and fungicides); Julius Hyman and Company, Denver, Colo. (Aldrin and dieldrin insecticides); John Bean Division, Food Machinery and Chemical Corporation, Box 840, Lansing, Mich. (Agricultural and automotive equipment, sprayers, and fog fighters); Standard Oil Company, 30 Rockefeller Plaza, New York 20, N. Y. (Spray Oils for every purpose); Aluminum Company of America, Pittsburgh 19, Pa. (Aluminum for all purposes); William Cooper and Nephews, Inc., 1909-25 Clifton Avenue, Chicago 14, Ill. (Manufacturing chemists); Rohm and Haas Company, Washington Square, Philadelphia 5, Pa. (Plastics, synthetic resins, and chemicals).

Chemical Insecticide Corporation, 57 13th Street, Brooklyn 15, N. Y. (Basic formulators for mosquito control); J. B. Williams Company, Glastonbury, Conn. (Mosquito repellents); Esso Standard Oil Company, 15 West 51st Street, New York 19, N. Y. (Spray oils for insecticides); Pest Control Equipment Company, 47 Forty-third Street, New York 18, N. Y. (Professional supplies for the pest control operator); Difco Laboratories, Inc., 920 Henry Street, Detroit 1, Mich. (Bactobrain heart infusion); John Powell and Company, Inc., 1 Park Avenue, New York 16, N. Y. (Basic materials for insecticide manufacturers); Ward's Natural Science Establishment, Inc., Box 24, Beechwood Station, Rochester, N. Y. (Professional supplies for the entomologist); Walton Laboratories, 1186 Grove Street, Irvington 11, N. J. (Humidifying and air condi-

tioning equipment); Bausch & Lomb Optical Company, Rochester 2, N. Y. (Optical products); Spray Engineering Company, 111 Central Street, Somerville 45, Mass. (Spraco nozzles); Essick Manufacturing Company, 1950 Santa Fe Avenue, Los Angeles, Cal. (Air cooling equipment, weed and pest control, and construction equipment); Chipman Chemical Company, Inc., Bound Brook, N. J. (Insecticides, fungicides, weed killers); E. I. du Pont de Nemours & Company, Wilmington, Del. (Insecticides, fungicides, weed killers, and auxiliary products); Monsanto Chemical Company, St.

Louis 4, Mo. (Organic chemicals); Ethyl Corporation, 100 Park Avenue Building, New York 17, N. Y. (Lindane, technical BHC (12 per cent-16 per cent gamma) and hi-gamma BHC (70 per cent-80 per cent gamma)).

ERNESTINE B. THURMAN

MYRTLE E. RUEGER

EMMA M. VICKERS

HELEN LOUISE TREMBLEY

HELEN SOLLERS

DOROTHY McCULLOUGH LEE

HARRY H. STAGE, *Chairman*,

Good Neighbor Club

ANOTHER SNIPE FLY PEST IN ALASKA. The importance of the biting snipe fly *Symphoromyia atripes* Bigot as a pest is recognized in many parts of subarctic Alaska. It is now necessary to point out that *S. kincaidi* Aldrich is equally important locally in Southeastern Alaska. *Kincaidi* appeared on the wing in one such area, Juneau, on June 22, 1952, a month before the first *atripes* were collected. Like *atripes*, this snipe fly not only bites man readily but repeatedly, since blood is engorged for about 10 per cent of human bites. It enters buildings to bite until blooded; those collected at windows are usually engorged. Attempts to hold these engorged females for oviposition in mosquito cages (foot-square) failed; few flies survived more than a day even when kept in the dark, covered with wet towels and given sugar sops.

Some intensive collecting by sweeping on the run was done in the Sheep Creek area near Juneau in the hope of settling the status of a form of *kincaidi* "with yellow pile all over," thought by Dr. Aldrich to be distinct. None of the yellow form was taken. However, 30 male *kincaidi* were collected, confirming Dr. Aldrich's association of the male which he, with reservations, assigned to *kincaidi*. Strangely enough, no male *atripes* has yet been taken, so far as known, in Alaska. W. C. Frohne, P.H.S., Anchorage, Alaska.

NEWS AND NOTES

DR. N. G. S. RAGHAVAN, Assistant Director of the Malaria Institute of India, is visiting various laboratories in the United States including those of the Bureau of Entomology and Plant Quarantine and the Public Health Service. He is studying work that is being done in connection with mosquito-borne diseases under auspices of the Point Four program. Dr. Raghavan is especially interested in filariasis.

AT THE COMMUNICABLE DISEASE CENTER of the Public Health Service, the Vector Control and Investigations Branch and the Technical Development Branch have been combined into a single organizational unit called the Technology Branch. Dr. S. W. Simmons is Chief of the new branch. Dr. George H. Bradley is now Chief Entomologic Consultant, Bureau of States Services, but he will remain in Atlanta. Mr. Chris A. Hansen has been named Assistant Officer in Charge of CDC.

NEWS FROM CALIFORNIA as reported in *Mosquito Buzz*:

Dr. Bernard Brookman has left the Encephalitis Laboratory in Bakersfield to join the Water Projects Section of the Public Health Service in Salt Lake City. Dr. R. Edward Bellamy is now in charge of the Bakersfield Encephalitis Laboratory of the Hooper Foundation.

Dr. Richard O. Hayes is now in Bakersfield working on problems connected with the measurement of mosquito populations as a joint project of the Public Health Service and the Hooper Foundation.

The Clear Lake gnat population has increased to the point where another treatment of the lake is planned for 1953, similar to the treatment made in 1949.

CAPT. FRANK G. FAVORITE has left the 3rd Army Medical Laboratory at Fort McPherson, Georgia for an assignment in the Far East.

DR. ARCHIE D. HESS is now in the Philippines where he is Chief Malariologist for the Mutual Security Administration. His address is MSA-STEM, APO 928, San Francisco.

DR. HERBERT A. CRANDELL is now doing malaria control work in Indonesia. His address is U. S. STEM, c/o American Embassy, Djakarta, Indonesia.

INTER-AMERICAN CONGRESS ON PUBLIC HEALTH. The First Inter-American Congress on Public Health was held in Havana, Cuba September 27 to October 1, 1952. The Congress marked the 50 anniversary of the Pan American Sanitary Bureau. Mr. H. H. Stage and the writer represented the American Mosquito Control Association. About 250 specialists in various branches of public health attended. Dr. Enrique Saladrigas

y Zayas, Minister of Public Health and Welfare of Cuba was elected president of the Congress.

Three main topics were discussed: (1) rural sanitation, (2) organization and integration of public health services, and (3) recent developments in disease prevention and control. Five symposia were held. These dealt with yellow fever, public health education, zoonoses, tuberculosis and maternal and child health. There were also round table discussions of malaria, chagas disease, schistosomiasis and onchocerciasis.

Much emphasis was placed on the control of vectors of malaria and yellow fever and the need for concerted international action against yellow fever by mosquito eradication and immunization.

Several delegates in stirring addresses paid tribute to Dr. Carlos J. Finlay and the important contribution he made to the yellow fever problem.

F. C. BISHOPP

THE SOUTH SALT LAKE COUNTY MOSQUITO ABATEMENT DISTRICT has been organized and is prepared to start mosquito abatement work in 1953. This district was created in 1951 and funds were made available in a 1952 tax levy. This is the fifth mosquito abatement district to be organized in Utah. Davis County which will probably be the sixth district, is still not officially organized although this county has been conducting a mosquito abatement program for the past three years on funds provided by the Board of County Commissioners.

THE SIXTH ANNUAL MEETING OF THE UTAH MOSQUITO ABATEMENT ASSOCIATION was held at Brigham City, Utah on February 21st, 1953, with the members of the Box Elder County Mosquito and Fly Abatement District serving as hosts to those attending. A program was prepared in which representatives of all of the abatement districts in Utah participated. Representatives of the State Association of County Officials, State Health Department, Water Projects Section of the Communicable Disease Center, Bureau of Recclamation, State Agricultural College, Weber College and University of Utah were also included on the program. Abstracts of the papers and reports presented will be prepared and distributed. Copies are available from Dr. A. W. Grundmann, Department of Invertebrate Zoology and Entomology, University of Utah, Salt Lake City, Utah.

DON M. REES

DR. LEWIS C. WEEKS, Commissioner of the Cape Cod Mosquito Control Project and member of the American Mosquito Control Association, a pioneer in mosquito control in Massachusetts, by the end of March will have completed his twentieth year of service as executive secretary to the largest mosquito control project in Massachusetts.

Although he retired from business and relinquished numerous other civic responsibilities in 1946, his interest in mosquito control at the same time became more intense, since he wished to see the project through the reorganization period which followed World War II. Needless to say, the State Reclamation Board, under which the project operates, and all other interested parties are very appreciative of Dr. Week's efforts in behalf of an agency which has been such an important factor in the prosperity of Cape Cod over the past twenty years.

B. I. Gerry

"A FEMININE ADVENTURER IN RESEARCH is Miss Helen Louise Trembley," according to a feature article in the WASHINGTON SUNDAY STAR, for Feb. 1, 1953. The occasion for this well-written and complimentary article was the appearance of Miss Trembley on the All-Star News Review, televised last December 7 over station WMAL. Besides giving a popular account of Miss Trembley's work, the article called attention to her connection with MOSQUITO NEWS and the AMCA.

A FACTOR INFLUENCING MALE MOSQUITO AND MIDGE SWARMS. While watching mating swarms of the mosquito, *Aedes punctor* (Kby.) in Southeastern Alaska recently, some observations were made which seem to indicate that the lightness of the surface under the swarm is important. Trampling down the tall grass under a swarm of males making a dark "hole" regularly resulted in a shift of the swarm which was flying several feet above the grass tops. Slipping a white bed sheet under a swarm broke it up at once. Thus mosquito swarms were broken up or shifted, but none was induced *de novo* in a prepared location.

The beautiful little tendipedid midge, *Anatopynia algens* (Coq.), kindly identified by Dr. Willis Wirth of the U. S. National Museum, proved easier to manipulate. Males of this species swarmed during sunset in the area over light-colored, flat objects such as weathered boards, shakes, or old newspapers. It was easy to induce a swarm of a hundred or so almost anywhere by spreading out the white bed sheet during the sunset swarming hours. Upon removal of the sheet the swarm at once broke up. When the sheet was moved up and down a 50 foot walk, it took its swarm along with it. Finally, three large "natural" swarms over less conspicuous light surfaces on the ground in a garden clearing were captured by the sheet. The males of one of these swarms were attracted from about 75 feet away. W. C. Frohne, P.H.S., Anchorage, Alaska.

EDITORIAL

The editorial page, again this month, is concerned with membership and subscription matters. Beginning on page 38 is printed a list of the members and their addresses as of March 1, 1953, the last date that the list could be held open.

Following the membership list is a list of persons and organizations who subscribe to the NEWS but are not members of the AMCA.

Attention is called to the fact that, owing to incompleteness of some of the records, each list may contain names which should have been classified in the other list. To those who are thus incorrectly classified we extend our apologies and request that they supply us with correct information.

There probably will have been several changes before the lists appear in print. This points up the fact that it is imperative to keep the Secretary informed of your whereabouts if you wish to receive your NEWS and other Association releases regularly. It takes a tremendous amount of work on the part of both the Secretary's office and the Editor's office to keep this list up to date.

Members are urged to look over the membership list and to use it frequently to keep in personal touch with other members.

The second list, also, deserves more than a passing glance from the members. Among other names, it includes those of many of our advertisers and suppliers, and their representatives — persons whose

names and addresses we should have on hand when we need something.

Of equal but perhaps more intangible importance, the list contains names and addresses of organizations which are officially interested in mosquito control, such as Armed Forces units, and medical research institutions and foundations, as well as of colleges, universities and libraries whose purpose and interests cover the whole field of education, research, and human advancement. It should be a source of satisfaction to AMCA members that their Association activities and the accomplishments of individual members as evidenced in their papers in MOSQUITO NEWS are of interest to these diverse groups throughout the world. It should stimulate the members to renewed effort to share their experiences and the results of their work, through MOSQUITO NEWS, with the increasing numbers of persons and groups which look to the AMCA for the latest information on mosquitoes and other insects of personal importance.

It should be pointed out also that as we increase the number of pages to accommodate more articles, we increase the cost of publication. To meet this cost we need continually to increase our membership and the subscription list. Let all AMCA members therefore bear in mind these two obligations: to do everything possible to increase the membership and subscription lists, and to contribute to the NEWS in such a way as to create the interest that makes this increase possible.

REVIEWS AND ABSTRACTS

THE MOSQUITOES OF THE NORTHWESTERN STATES. Stage, H. H., Gjullin, C. M., and Yates, W. W. USDA Agriculture Handbook No. 46. November 1952, 95 pp. This is a similar volume to Miscellaneous Publication 336, "The Mosquitoes of the Southeastern States." It treats of the very interesting mosquito fauna of Washington, Oregon, and Idaho, and includes thirty-nine species.

The first half is devoted to a general discussion of the importance, collecting techniques, and control of species concerned. This section is thoroughly justified by the ecological problems involved with flood- and snow-water breeding, which are unique to this area and a few surrounding states.

The latter half contains excellent keys, divided by genera which separate the species by adults, male genitalia and by larval characteristics. Each species is treated separately, with a concise description of the adult female, the male genitalia, the larva and an informational note on biology, distribution and importance. In addition there are splendid distributional maps that add a great deal to the publication, and a bibliography of 175 titles.

The authors have made a splendid contribution that will be a well-thumbed volume in the library of all entomologists in the area, and a valuable guide and reference for mosquito workers everywhere.

The only criticism is that the absence of adequate explanatory drawings of larval characters will make the intelligent use of the keys a bit difficult for the novice.—Stanley B. Freeborn, Univ. Calif., Davis, Calif.

MOSQUITO FAUNA OF NORTH AMERICA. Satyu Yamaguti and Walter J. LaCasse, Office of the Surgeon, Headquarters Japan Logistical Command, APO 343. This monograph is divided into 5 parts: I, Genus *Anopheles*; II, Genera *Megarhinus*, *Wyeomyia*, *Uranotaenia*, and *Culiseta*; III, Genera *Orthopodomyia*, *Mansonia*, and *Psorophora*; IV, Genera *Culex* and *Deinocerites*; and V, Genus *Aedes*. Descriptions, keys to adult females, male terminalia, and fourth instar larvae are included all with great emphasis on accurate and detailed drawings for easy identification. There are 13 species of *Anopheles*, 2 of *Megarhinus*, 4 of *Wyeomyia*, 4 of *Uranotaenia*, 7 of *Culiseta*, 3 of *Orthopodomyia*, 3 of *Mansonia*, 12 of *Psorophora*, 26 of *Culex*, 2 of *Deinocerites*, and 58 *Aedes* included. Copies of the monographs are now available only to the military, but it is anticipated that a slightly enlarged edition will be printed and released by the Department of Defense late in 1953.—H. H. Stage.

NORTHERN BRITISH COLUMBIA AND THE YUKON. By J. F. Sharp. Canadian Entomologist 84(9): 281–291. 1952. Experimental area control of adult mosquitoes by airspray was carried out at four stations of the R.C.A.F. in the North West Air Command during the summer of 1950. Approximately 42,240 acres (66 sq. mi.) were treated with 66 drums of 30 per cent DDT concentrate in Velsicol AR-50 and 30,000 gal. of fuel oil; three quarts per acre of a 3.5 per cent DDT solution gave a coverage of 0.23 lb. of DDT per acre.

Approximate percentage control at the stations as determined by biological assessments of the spray plots before and immediately after the application was as follows: Whitehorse, 1st spray; 93, 2nd, 98; Watson Lake, 99; Fort Nelson, 1st spray, 64, 2nd, 70.

At Whitehorse, Watson Lake, and Norman Wells good control was maintained during the season. The first spray at Fort Nelson gave fair control for about two days and the second for seven days, after which the mosquito population decreased to negligible numbers in both treated and untreated areas.—Author's summary.

REACTIONS OF *Aedes aegypti* (L.) TO CARBON DIOXIDE. By Edwin R. Willis and Louis M. Roth. J. Exp. Zool. 121(1):149–179. 1952. Air containing 0.1 per cent to 50 per cent CO₂ moderately repelled all adult *Aedes aegypti* of both sexes when tested in groups in a small-cage olfactometer in the dark at 27° and 33° C., but when they were tested in a large-cage olfactometer at 23°–25° C. participating mosquitoes (less than 10 per cent of the test population) were positively attracted to an air stream containing 10 per cent CO₂. Those which made a choice between air alone and air with CO₂ (within the large-cage olfactometer) preferred 10 per cent CO₂. When 100 adults were tested individually in the small-cage olfactometer, 6 of them had "O" reaction and 18 of them were not repelled by the CO₂, 6 of those not repelled were possibly positively attracted by the gas. The above reactions were not significantly altered by contrasting differences in light, temperature, humidity, age, or blood feeding. The authors suggest either that CO₂ is a potential attractant for female mosquitoes and that they vary in their sensitivity to it, or that there are two distinct types of adults: those attracted and those repelled or indifferent.

The authors attribute the differences in directional reactivity of adults to CO₂ within the two types of olfactometers to a difference in proportions of total numbers participating in test reactions, since all participated in the small cage but less than 10 per cent did so in the large-cage olfactometer.

Discrimination of CO₂ by adult mosquitoes is

due to olfactory stimulation of unidentified receptors on the first flagellar segments of the antennae.—Jack Colvard Jones, National Institutes of Health, Bethesda, Md.

ANNUAL REPORT, Malaria Division, Government of Trinidad and Tobago, 70 pp., H. P. S. Gillette, 1952, Processed. The report continued to show a marked decline of malaria in the islands. Compared to the 600 deaths caused by this disease in 1943 there were only 136 reported during 1951. An examination of 9,474 school children showed a spleen rate of 2 per cent. A study was reported on the bionomics of *Anopheles aquasalis* in which its breeding habits in mangroves and ricefields, outdoor resting habits, and anthropophilic index was emphasized. Some 600 additional acres were sprayed with 0.5 per cent copper sulfate in controlling bromeliads, an indirect control of *Anopheles bellator*.

The DDT residual spraying on Trinidad was extended to 27,958 homes in which nearly 160,000 people were protected against malaria. Every house was sprayed on Tobago, once with DDT and once with BHC. The spleen rate on this island was 0.8 per cent of 3,470 school children. Preliminary results were given on the use of a 25 per cent wettable dieldrin powder as a larvicide. An application of 0.12 pound of dieldrin per acre secured control of anopheline breeding for more than 14 days. Fifty houses built with adobe were protected for 5 months with dieldrin residual sprays.—H. H. Stage.

MOSQUITO-REPELLENT MIXTURES. By C. N. Smith, M. M. Cole, G. W. Lloyd, and A. Schiime. Jour. Econ. Ent. 45(5):805-809. 1952. Of the thousands of chemicals that have been tested by various agencies as mosquito repellents, less than a score have proved highly effective and also safe for unrestricted application to the skin. Experience has shown that mixtures of several good repellents are effective against a wider range of species and on more individuals than any one repellent used alone. Studies during World War II culminated in the selection of a mixture (M-250), consisting of 6 parts of dimethyl phthalate, 2 parts of 2-ethyl-1, 3-hexanediol, and 2 parts of Indalone, for general use by the armed forces. Experiments have been continued to find other mixtures or individual repellents, of a higher general effectiveness than M-250. As in previous observations, the best over-all results were obtained with mixtures. The most effective mixtures against several species of *Aedes* were dimethyl phthalate 4 parts, 2-ethyl-1, 3-hexanediol 3 parts, dimethyl carbate 3 parts (M-2020); dimethyl phthalate 4 parts, 2-ethyl-1, 3-hexanediol 3 parts, propyl N, N-diethylsuccinamate 3 parts (M-2043); 2-ethyl-1, 3-hexanediol 4 parts, propyl N, N-diethylsuccinamate 3 parts, O-chloro-N, N-diethylbenzamide 3 parts (M-2042); and 2-ethyl-1, 3-hexanediol 5 parts, O-chloro-N, N-diethyl benzamide 5 parts (M-2048).

Several of the most effective mixtures against

Aedes mosquitoes proved relatively ineffective against *Anopheles quadrimaculatus*.—Authors' summary.

IRRIGATION AND MOSQUITOES IN THE UNITED STATES OF AMERICA. By John M. Henderson. Indian Jour. Malariology. 6(1):23 pp. 1952. 29 refs., 10 tables. The author mentions by way of introduction his detail to the Malaria Institute of India in 1949, while serving as consultant on malaria control with the World Health Organization, assigned principally to field surveys of irrigation projects under construction. Subsequently, he made an extended field study of irrigation design and irrigation practices in the U.S.A. while serving as consultant to the Communicable Disease Center, U. S. Public Health Service.

This request article is intended to apprise readers in India who are engaged in irrigation or public health activities with irrigation design and practices in the U.S.A. from the standpoint of mosquito production.

The first portion of the paper presents the disease and nuisance aspects of irrigation-mosquitoes in the U. S., past and present. This is followed by a history of irrigation and a description of irrigation methods and modern principles of water application in the U. S. Irrigation in India and other parts of Asia up to the present has been principally "flooded-field" irrigation, generally for rice cultivation. Most irrigation in the U. S. is "dry-crop" irrigation, as it is termed in India. Because current large scale irrigation projects in India provide for "dry-crop" irrigation, the article mainly discusses irrigation for this purpose.

The remainder of the article discusses in detail the types of mosquito-breeding places encountered in irrigated areas, irrigation practices which directly cause them, and the indirect socio-economic factors which increase or lessen mosquito production through their influence on irrigation development and practices. These include: carelessness and inattention, (resulting in part from the dynamic character of irrigation-agriculture and high manpower productivity), conservation-irrigation, irrigation economics, speculative farming, land tenancy, age of irrigation development and water-rights.

In conclusion, the author points out that the most significant factor in preventing "irrigation-mosquito" production under "dry-crop" irrigation is "that the practices which prevent mosquito production largely are also good agricultural and irrigation practices from which farmers receive benefit in the form of improved crop yields, lowered water costs, or both." In view of the magnitude of the malaria problem in India (100 million cases annually), much of which is derived from irrigation, measures of this type are considered essential there and in many other parts of the world, in spite of the effectiveness of DDT and related insecticides.—John M. Henderson, Columbia Univ. Sch. Public Health, and Communicable Disease Center, Savannah, Ga.

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LIST OF MEMBERS OF AMERICAN MOSQUITO CONTROL ASSOCIATION, INC.

(See Editorial Page for comments and explanation)

A

- Aamodt, T. L., 300 Adm. Bldg., Univ. Farm, St. Paul, Minn.
 Aarons, Theodore, Alameda Co. Mosq. Ab. Dist., Cour House, Oakland 7, Calif.
 Abramitis, Walter W., 4734 Belmont Rd., Downers Grove, Ill.
 Affronti, Lt. Lewis F., Med. Entomologist, USAF, MSC, Box 598, Patrick Air Force Base, Florida.
 Aitken, Thomas H. G., The Rockefeller Foundation, 49 W. 49th St., New York 20, N. Y.
 Alrutz, Robert W., Denison University, Granville, Ohio
 Altman, Capt. R. W., U. S. Army Medical Corps, P. O. Box 3391, Orlando, Florida
 Alvarado, Dr. Carlos Alberto, Av Mitre 569, Tucuman, Argentina, S. A.
 Alvarez, Ing. Pedro M., Estacion De Malaria-logia, Acarigua, Venezuela
 American Entomological Society, % The Academy of Natural Sciences of Philadelphia, 19th and the Parkway, Philadelphia, Pa.
 Anderson, C. E., 95 Hanover Ave., Morris Plains, N. J.
 Anderson, Earl D., Sec., Nat'l Sprayer & Duster Ass'n, 141 W. Jackson Blvd., Chicago 4, Ill.
 Anderson, Mrs. Florence T., Morris Co. Mosq. Ext. Comm., Morris Plains, N. J.
 Andronico, Matthew, 68 Rutgers Place, River Edge, N. J.
 Ardo, Paul, Assistent, Fil. Kand. Zool. Inst., Lund, Sweden
 Arendt, Wm. H., 40 W. Johnson St., Phila. 44, Pa.
 Armstrong, Robert F., E. Middlesex Mosq. Cont. Project, City Hall, Cambridge, Mass.
 Arnold, Frank D., 1202 Mission Road, Salt Lake City, Utah
 Arnold, John R., 834 W. Mariposa, Stockton, Calif.
 Askwith, Winston Moore, Federal Dist. Comm., 291 Carling Ave., Ottawa, Canada
 Auguston, G. F., Mgr., Madera Co. M A D, P. O. Box 898, Madera, Calif.
 Austin, Herbert F., Grove Ave., Patchogue, L. I., N. Y.

B

- Baker, Walter C., U. S. Stem, % American Legation, Saigon, Viet-Nam
 Barnard, Lenham T., Safety Engr., Brown Pacific Maxon, Station 1, Guam, Guam
 Barnes, Dr. D. Keith, Co. Health Supervisor, Kaysville, Utah
 Barnes, Ralph C., USPHS, CDC, 50 Seventh St., N.E., Atlanta, Ga.

- Barr, Allan R., University of Minnesota, University Farm, St. Paul 1, Minn.
 Bartlett, Frank B., Town of Cranberry Isles, Islesford, Maine
 Barnett, Robert Elliott, Dir., Anastasia Post Office, St. Augustine, Florida
 Basnuevo, Dr. Jose, University Habana, Apartado 6 Fo, Habana, Cuba
 Beadle, Leslie D., Mitchell Field Station, Pathfinder Irrigation Bldg., Mitchell, Nebraska
 Beaton, Norman S., USPHS, Hilton Village, Va.
 Beckel, William E., Ent., D.R.N.L., Fort Churchill, Manitoba, Canada, M. P. O. 1015
 Becton, Edward M., Indian River Mosq. Control Bd., Box 884, Vero Beach, Fla.
 Berksan, N. A., M.D., Dept. of Medicine, University of Chicago, Chicago 37, Illinois
 Bertholf, James H., Dir. Broward Co. Anti-Mosq. Dist. Court House, Ft. Lauderdale, Fla.
 Berti, Arturo Luis, Div. de Malaria-logia, Maracay, Venezuela
 Besozzi, Leo, 314 Hammond Bldg., Hammond, Ind.
 Bickley, William E., Assoc. Prof. Entomology, University of Maryland, College Park, Md.
 Bidwell, Warren, Seaview Country Club, Abscon, N. J.
 Bishopp, Dr. F. C., USDA, Bur. of Entomology, Washington, D. C.
 Bitting, John J., 108 Green St., Woodbridge, N. J.
 Black, Harold, Mercer Co. Mosquito Ext. Comm., Court House, Trenton, N. J.
 Blair, George A., Buttonwood Dr., Shrewsbury, N. J.
 Blakeslee, Capt. Theodore E., USA, 2nd Army Area, Med. Lab., Ft. George G. Mead, Md.
 Blancarte, Dr. Juan Ramon, Escobedo 138 Pte, Culiacan Sin, Mexico
 Blank, Walter, Pres., Abalene Pest Control Service, 70 Market St., Poughkeepsie, N. Y.
 Blanton, Lt. Col. Franklin S., USA, 25th Prev. Med. Survey Det., Ft. Clayton, Canal Zone
 Blum, Richard A., Sen Inspector, Bergen Co. Mosq. Comm., E-108 Linwood Ave., Paramus, N. J.
 Bohart, Richard M., Div. Entomology, Univ. Calif., Davis, Calif.
 Boyd, Dan F., Vice-Pres., Southern Mill Creek Products Co., Inc., 214 Greyhound Bldg., Tampa, Florida
 Bradley, Clarence L., San. Fargo Health Dept., 6 Roberts St., Fargo, N. Dakota
 Bradley, George, Public Health Service, 50 Seventh St., N.E., Atlanta 5, Ga.
 Bradley, William G., Res. Advisor in Entomology, American Embassy, Ciudad Trujillo, Dominican Republic

- Brady, Frank J., NSMAD, 1275 Scott Ave., Hubbard Woods, Ill.
- Brawley, John H., Hanford Mosq. Ab. Dist., P. O. Box 303, Hanford, Calif.
- Bray, James H., Bur. Vector Control, 334 Rowell Bldg., Fresno, Calif.
- Breland, Osmond P., Univ. of Texas, Austin, Tex.
- Bridges, C. Bradley, USPHS, P. H. S.—MSA, APO 206A, % PM, New York, N. Y.
- Brocco, Eude, Estacion De Malariologia, Maracaibo, Venezuela
- Brockway, Porter Bruce, Jr., Toledo Area San. Dist., 5015 Stickney Ave., Toledo 2, Ohio
- Bronson, A. H., 555 So. Flower St., Los Angeles 17, Calif.
- Brookman, Dr. Bernard, U. S. Public Health Service, P. O. Box 386, Salt Lake City, Utah
- Brooks, Gerald D., Tulare Mosq. Ab. Dist., 467 So. Larkin St., Tulare, Calif.
- Brown, A. W. A., Prof. Zoology, Univ. of Western Ontario, London, Ontario, Canada
- Brown, Dr. V. E., Asst. Prof. of Biology, Marquette University, Milwaukee, Wisconsin
- Browne, Andrew C., 1200 Woodland Ave., Palo Alto, Calif.
- Bryan, Capt. H. John, 0924848 Det. D., 7806 SCU APO 162, % Postmaster, New York, N. Y.
- Bullard, J. Preston, Desplaines Valley Mosq. Ab. Dist., 8130 Ogden Ave., Lyons, Ill.
- Bullock, Howard R., Division of Biology, B 304, University of Utah, Salt Lake City, Utah
- Bumiller, E. J., 2426 Sleepy Hollow Drive, Glendale, Calif.
- Bunn, Lt. Col. Ralph W., 5510 Charlcote Rd., Bethesda 14, Md.
- Burger, Louis, American Extermin. Co., Inc., 341 Washington Ave., Albany, N. Y.
- Burton, Dr. George J., Ento. Sanitary Engineering Branch, Engineer Research & Development Lab., Fort Belvoir, Va.
- Bussart, J. Everett, Ent., The Velsicol Corp., 215 W. Harrison St., Wheaton, Ill.
- Butcher, Fred D., Ent. HQ, AFPE, APO 343, % Postmaster, San Francisco, Calif.
- Butts, Dr. Donald C. A., 3800 S. W. 58 Court, Miami 44, Fla.
- Buxton, Jay A., Dept. of Zoo. & Ent., Ohio State Univ., Columbus 10, Ohio
- Byrd, George M., Calif. St. Dept. of Pub. Health, 334 Rowell Bldg., Fresno, Calif.
- Carrillo, Salvador Jose, Div. de Malariologia, Maracay, Venezuela, South America
- Carroll, Edgar L., 133 E. Taylor Ave., Phoebus, Va.
- Carter, Richard G., Supt., Va. Beach-Princess Anne Co. Mos. Control Comm., Town Hall, Va. Beach, Va.
- Castillo, Dr. Roberto Levi, P. O. Box 3606, Guayaquil, Ecuador, S. A.
- Cave, Douglas, Weber Co. Mosq. Ab. Dist., 2640 Liberty Ave., Ogden, Utah
- Charles, Dr. L. J., Chief Officer, Mosquito Control Service, British Guiana Govt. Med. Dept., Georgetown, British Guiana
- Chen, Teh-Neng, Taiwan Agric. Research Inst. Taipei, Taiwan (Formosa), China
- Chester, Burke, Lake County Health Dept., Tavares, Fla.
- Christopher, Ralph, Weedsport, N. Y.
- City of Portland 45453, Bur. of Insect Control, 6525 S. W. Capitol Hill Road, Portland, Oregon
- Clarke, J. Lyell, Desplaines Valley Mos. Abat. Dt., Lyons, Illinois
- Coburn, William S., County Executive, P. O. Box 411, Hampton, Va.
- Cochran, Alexander M., 56 Greenwood Ave., Montclair, N. J.
- Coleman, Richard D., Dept. of Industrial Health, Harvard School of Pub. Health, 55 Shattuck St., Boston 15, Mass.
- Collins, Dr. Donald L., Sci. Service, N. Y. State Education Bldg., Albany 1, N. Y.
- Collins, Roi C., 126 Park St., Westfield, N. J.
- Colvin, Timothy E., Pres., Besler Corp., 4053 Harlan St., Emeryville 8, Calif.
- Conant, Vernon, Bergen Co. Mosq. Ext. Comm., 420 Administrative Bldg., Hackensack, N. J.
- Conley, Thomas M., Halco Chemical Co., Kenilworth, N. J.
- Contra Costa Mosquito Abatement Dist., P. O. Box 447, Martinez, Calif.
- Cory, Dr. E. N., University of Maryland, College Park, Maryland
- Cotta, William J., Mansfield Ave., Darien, Conn.
- Covell, General Sir Gordon, Dir. Malaria Lab., Horton Hospital, Epsom, Surrey, England
- Cox, Wm. M., Galveston Mos. Con. Program, City Hall, Galveston, Tex.
- Craig, George B., Jr., 10101 S. Hoyne Ave., Chicago 43, Ill.
- Crandell, Herbert A., TCA Mission To Indonesia, Department of State, Washington 25, D. C.
- Crawford, G., 8130 Ogden Ave., Lyons, Ill.
- Creadon, Francis P., Desplaines Valley Mosq. Ab. Dist., 8130 Ogden Ave., Lyons, Ill.
- Crowell, H. B., Jr., Hillsborough Co. Health Dept., 1420 Tampa St., Tampa, Fla.
- Cuadrado, Dr. Jose Gamboa, Estacion de Malariologia, Este 8, No. 217, Los Caobos, Caracas, Venezuela, S. A.
- Cummings, Harold N., 695 Grove St., Upper Montclair, N. J.
- Curran, C. H., Amer. Mus. Nat. History, 15 W. 77th St., New York 24, N. Y.

D

Dahl, Arve H., Chief San. Sec., State Office of Civil Defense, 839 Phelan Bldg., 760 Market St., San Francisco 2, Calif.

Dalmat, Herbert T., USPHS, Chief Onchocerciasis Proj., Oficina Sanitaria Panamericana, Apt. 383, Guatemala, C. A.

Darsie, Richard F., Jr., Agric. Expt. Station, University of Delaware, Newark, Del.

Davies, Dr. Douglas M., Dept. Zoology, McMaster Univ., Hamilton, Ontario, Canada

Davis, Byron G., M.D., 1515 Pacific Ave., Atlantic City, N. J.

Davis, E. D., Mgr., Fresno Mosq. Ab. Dist., P. O. Box 2, Fresno, Calif.

Davis, Franklin B., Route 1, Box 611, Ceres, Calif.

Davis, Harry R., 711 North Fifth St., Boise, Idaho

Davis, Roscoe C., Cal. St. Dept. Pub. Health, 703 State Bldg., Los Angeles, Cal.

Davison, R. H., Town of Truro, Nova Scotia

De Brava, Fred, 97 Hillside Park Drive, New Hyde Park, N. Y.

De Bustamente, Dr. Fernando M., R. Aristides Espinola 11, Apt. 3, Leblon, Rio De Janeiro, Brazil, S. A.

Decaires, P. F., Constant Spring P. O., St. Andrew, Jamaica, B.W.I.

DeCoursey, John D., CDR, MSC, USN, Naval Med. Field Res. Lab., Marine Barracks, Camp Lejeune, N. C.

Defoliart, Gene R., Asst. Ent., Entomology Dept., Univ. of Wyoming, Laramie, Wyo.

Gauna, Fernando De Guinea, Div. De Malariologia, San Fernando de Apure, Venezuela

Del Rosario, Fidel G., Sc.D., 150 P Burgos St., Cavite City, Cavite, Philippines

Dengler, Dr. Henry P., 260 Morris Ave., Springfield, N. J.

Denning, Dr. D. G., Velsicol Corp., 2082 Center St., Rm. 410, Berkeley 4, Calif.

Dennis, J. W., Jr., 407 E. Swells Point Rd., Norfolk 5, Va.

Derosé, Charles R., Box 52, Adamstown, Maryland

Diaz, Ramon Gayosa, Div. De Malariologia, Carupano, Venezuela

Dillistin, Wm. H., 443 E. 39th St., Paterson, N. J.

Director of Medical Services, Kingston, Jamaica, B.W.I.

Dir., Science Service Lab., Canada Agriculture, University Sub Post Office, London, Ontario, Canada

Doane, Oscar W., Jr., Cape Cod Mosq. Con. Proj., Colonial Acres, Hyannis, Mass.

Doane, Oscar W., Sr., George Ryder Road, West Chatham, Mass.

Dodge, Albert W., The Dodge Associates, Wenham, Mass.

Dolan, D. D., President, Dolan Laboratories, Inc., 7370 Dale Ave., Clayton, Missouri

Dominion Entomologist, Dept. of Agriculture, Confederation Building, Ottawa, Ontario, Canada

Dorer, Rowland E., 300 Essex Bldg., Norfolk, Va.

Doughty, Frank M., Madison Ave., Plainfield, N. J.

Dow, Richard P., Box 270, Thomasville, Ga.

Downs, Dr. Wilbur G., Trinidad Regional Virus Lab., P. O. Box 164, Port of Spain, Trinidad

Drake, Dr. C. J., Entomologist, Iowa State College of Agric., Ames, Iowa

Du Chanois, Lt. F. R., MSC, USN, U S N Preventive Med. Unit 6, Navy No. 128, FPO, San Francisco, Calif.

Duclos, W. Earle, 862 W. Santa Barbara, Los Angeles 37, Calif.

Dunney, Wm. P., 50 William St., Clifton, N. J.

Dwyer, Henry E., 35 Pleasant Ave., Passaic, N. J.

Dy, Dr. Francisco J., WHO Regional Office, 25th St., Port Area, Manila, Philippines

E

Eager, George, 129 No. Walnut St., East Orange, New Jersey

Edgar, Dr. S. Allen, Poultry Department, Alabama Polytechnic Inst., Auburn, Alabama

Edmunds, Lafe R., Asst. Sanitarian, USPHS, Pathfinder Irrigation Bldg., Mitchell, Nebraska

Ehrenberg, Herman, Foreman, Bergen Co. Mosq. Comm., 9 West Century Rd., Paramus, N. J.

Ehrlich, Alexander, P. O. Box 1435, Lancaster, Pa.

Eichler, Wm. A., Velsicol Corp., 330 E. Grand Ave., Chicago 11, Ill.

Elishewitz, Dr. H., The Chicago Med. School, 710 So. Wolcott Ave., Chicago 12, Ill.

Elston, Julius, Chief, Div. of Mosquito Control, Conn. State Dept. of Health, P. O. Box 708, Madison, Conn.

Emerson, Don, Dir. Mosquito Control, City of Lakeland, City Hall, Florida

Esmeja, Dr. Pedro Kouri, 5a Avenida y Calle 78, Reparto Playa de Miramar, Habana, Cuba

Essertier, Dr. Edward P., 273 State St., Hackensack, N. J.

Evans, Sterling, Pres., Evans Orchard Supply Co., 305 Delaware, Kansas City 6, Mo.

F

Fales, John H., Entom. USDA, Bur. of Entom. & Plant Quar. USDA, Bldg. A, U. S. Dept. Agr. Res. Center, Beltsville, Md.

Falkner, Mack D., NSMAD, 8712 Callie Ave., Morton Grove, Ill.

Farr, Shirley, Brandon Org. for Mosq. Control, Brandon, Vt.

Fathy, Dr. Abdel Aziz, Chief of Insecticides Div., Public Health, 2 Koraiun Shorek St., Apt. 7, Giza, Egypt

Favorite, Frank G., 3425 Tulane Dr., Apt. 33, West Hyattsville, Maryland

Feenan, Joseph, 255 Grand St., Paterson, N. J.

Fellton, Herman L., Orkin Exterminating Co., Inc., 590 Courtland St., Atlanta, Ga.

Field, Gordon, Preventive Medicine Div., Office of the Surgeon General, The Pentagon, Washington 25, D. C.

Finley, C. C., Field Supt., Magna MAD, Box 112, Magna, Utah

Fisher, William A., 115 Ridge Road, North Arlington, N. J.

Fleenor, Ernest B., Socony Vacuum Oil Co., 10 Light St., Baltimore, Md.

Fletcher, E. H., Aero-Flight Co., Troutdale Airport, Troutdale, Oregon

Floch, Dr. Herve, Dir. de l'Institut Pasteur, Cayenne, Guyana, France

Fluno, John H., Entomologist, Bur. Ent. & Pl. Quar., U. S. Dept. of Agriculture, 245 Sylvan Blvd., Winter Park, Fla.

Flynn, John, Supt., Passaic Co. Mosq. Ext. Comm., Paterson, N. J.

Foley, Lt. Paul M., Commanding Officer, 547th Prev. Med. Survey Det., APO 757, % PM, New York, N. Y.

Fontaine, Russell B., Calif. St. Dept. Pub. Health, 631 J St., Sacramento, Calif.

Foot, Richard H., Div. of Insect Identification, U. S. Dept. of Agriculture, Washington 25, D. C.

Foulk, Charles T. II, 150 Overlook Terrace, Bloomfield, N. J.

Fox, Dr. Irving, School of Tropical Medicine, San Juan, Puerto Rico

Frank, Lewis E., Dir. Weber Co. Mosq. Ab. Dist., 505 W. 12 St., Ogden, Utah

Freeborn, Dr. Stanley B., Provost, University of California, Davis, Calif.

Freytag, Henry E., 160 Northern Parkway, East Orange, N. J.

Friedman, Herbert J., Pres., Southern Mill Creek Prod. Co., 214 Greyhound Bldg., Tampa, Fla.

Fritz, Roy F., USPHS, CDC, 50 Seventh St., N.E., Atlanta 5, Ga.

Frohne, William C., USPHS, Box 960, Anchorage, Alaska

Furrow, Lucian M., U. S. Engineers Office, Norfolk 1, Va.

G

Gabaldon, Arnaldo, Div. de Malariologia, Maracay, Aragua, Venezuela

Gahan, James B., 413 Anderson St., Orlando, Fla.

Gardella, Louis, Jr., R.S., District Sanitarian, Trust Territory of Pac. Isles, Truk, East Caroline Islands

Gates, Ralph, 364 Hartshorn Drive, Short Hills, N. J.

Geb, A. F., Route 1, Box 722, Arvin, Calif.

Geisman, Joseph L., 219 Monticello Ave., Jersey City 4, N. J.

Gerberg, Eugene J., Insect Cont. & Research, Inc., Johnnycake Road, Baltimore 7, Md.

Gerberich, John B., Insect Control Res. Products, P. O. Box 21, Duluth, Minn.

Gerhardt, Richard W., 4921 Pennsylvania Ave., Fair Oaks, Calif.

Gerlach, Charles F., Michigan Chem. Corp., Saint Louis, Mich.

Gerry, Bertram I., State Reclamation Board, 41 Tremont St., Boston 33, Mass.

Getz, Carl W., D. B. Smith & Co., N. Broad St., Lansdale, Pa.

Gibson, Jonathan S., 12 River Road, Hilton Village, Va.

Gies, Russel W., Delaware Co. Mosq. Ext. Comm., Court House, Media, Pa.

Gilbert, George C., Box 172, Kingston, N. J.

Giles, James A., 228 First Ave., Apt. 14, Salt Lake City 3, Utah

Gill, Wm. H., Dept. Pub. Health, 108 W. Cary St., Richmond, Va.

Gillette, Dr. H. P. S., P. O. Box 556 Port of Spain, Trinidad, B.W.I.

Ginsburg, Dr. J. M., N. J. Agr. Expt. Sta., New Brunswick, N. J.

Gjullin, C. M., Bureau Entom. & Plant Quar., Corvallis, Ore.

Goddard, Mrs. George, 125 E. Maple St., Lombard, Illinois

Good, Newell E., USPHS, 50 Seventh St., N.E., Atlanta, Ga.

Goulding, Robert L., Jr., 2723 Washington St., Hollywood, Fla.

Graham, Jay E., Box 26, Sugarhouse Station, Salt Lake City, Utah

Granados, Juan A., Com. de Malaria de Cuba, Apartado 157, Habana, Cuba

Grannet, Dr. P., N. J. Agr. Expt. Sta., New Brunswick, N. J.

Grant, C. Donald, Mgr.-Entom., Pulgas-Three Cities M.A.D., 865 Sherman Ave., Menlo Park, Calif.

Graves, J. R., P. O. Box 922, Vero Beach, Fla.

Gray, Harold F., 1A Court House, Oakland 7, Cal.

Griffith, Melvin E., Chief Malaria Control Adviser, USA-Stem to Thailand, % American Embassy, Thailand

Griffiths, A. E., 10 Madison St., Port Washington, N. Y.

Grundmann, Dr. Albert W., 2374 Sheridan Rd., Salt Lake City, Utah

Gulden, Frank, Bayberry Point, Islip, L. I., N. Y.

H

Hagmann, Lyle E., Rutgers University, New Brunswick, N. J.

Hallowell, Charles K., Co. Agent, 303 Drexel Bldg., Philadelphia, Pa.

Hanna, Ralph B., Jr., 1615 LaSalle Ave., Norfolk, Va.

Hansen, Chris A., USPHS, CDC, 50 7th St., Atlanta, Ga.

Hardy, Karl D., 373-7th East St., Salt Lake City, Utah

Hare, Frank J., Le Hava Air Services, 258 So. Van Pelt St., Phila. 3, Pa.

Harmstrom, Fred C., 4120 Margaretta Ave., St. Louis 15, Missouri

Harrison, Zadok D., 835 Bonifant St., Silver Spring, Maryland

Hathaway, Prof. E. S., Tulane University, New Orleans, La.

Hathaway, John S., M.D., Institute of Inter-American Affairs, Div. of Health, Welfare & Housing, % American Embassy, Lima, Peru

Hayes, Harold B., Jr., State Supervisor-Insect Cont., Georgia Dept. of Public Health, P. O. Box 1095, Macon, Ga.

Hayes, Richard O., 1108 Lincoln St., Bakersfield, Calif.

Hedeen, Lt. Robert A., Dept. of Zoology, University of Texas, Austin, Texas

Heidt, James H., Dade Co. Mosq. Dist., Box 274, Dania, Fla.

Heinrichs, Wm. C., Pres., Highland Park Mosq. Ab. Dist., 685 Park Ave., Highland Park, Ill.

Helm, Robert W., Rice Experiment Station, Crowley, La.

Henderson, John M., USPHS, % Communicable Disease Center, P. O. Box 769, Savannah, Ga.

Henderson, Walter D., Ex. Sec., Ocean Co. Mosq. Ext. Com., Barnegat, N. J.

Hendrickson, Ole J., 936 38th St., Sacramento 16, Calif.

Hess, Dr. A. D., Mas-Stem, APO 928, % Postmaster San Francisco, Calif.

Hewitt, J. E., Executive Dir., Lebanon Chemical Co., Farmer's Trust Bldg., Lebanon, Pa.

Hill, Arthur M., Jr., P. O. Box 306, Vero Beach, Florida

Hill, Lt. Col. Samuel O., M.F.S.S., Ft. Sam Houston, Texas

Hines, E. R., Sec.-Treas., Kempsville Mosq. Con. Comm., RFD 1, Box 321, Bayside, Va.

Hinman, Dr. E. H., Dean, School of Medicine, University of Puerto Rico, San Juan 22, Puerto Rico

Hirst, Lt. Cdr. John M., MSC, USN, U. S. Naval Air Station, Alameda, Calif.

Hocking, B., Asst. Prof. Entom., Univ. of Alberta, Edmonton, Alberta, Canada

Hodapp, Lt. Cyril J., A02228952, 4th Epidemiological Flt., USAFE, APO 633, % Postmaster, New York, N. Y.

Hoff, Joseph S., 45 Wiggins St., Princeton, N. J.

Hoffman, James L., 1414 S. Float Ave., Freeport, Ill.

Hoffman, Robert A., Entomologist, BEPQ, USDA, Box 332, Corvallis, Oregon

Holbrook, Gerald C., Prouts Neck, Maine

Hollyer, Richard V., Pres., Fog Inc., Godwin Ave., Wyckoff, N. J.

Holmes, Jos. G., Sales Mgr., Snowden Chemical Co., P. O. Box 1213, Modesto, Calif.

Howse, B. C., P. O. Box 537, Fairfield, Ala.

Hu, Stephen M. K., Sc.D., Bureau of Mosquito Control, Dept. of Health, Honolulu 1, Hawaii

Huber, John H., 3425 N. H. 40 Ave., Portland 13, Oregon

Husbands, Richard C., 2645 Flora, Fresno 3, Calif.

Husman, C. N., USDA, Box 3391, Orlando, Fla.

Hutchcraft, J. R., Office of Dist. Med. Officer, Headquarters 6th Naval Dist., U. S. Naval Base, Charleston, So. Carolina

Hutton, George L., Supt., Toledo Area San. Dist., 5015 Stickney Ave., Toledo 12, Ohio

I

Institute of Inter-American Affairs, Avenida Rio Branco 251-12, Caixa Postal 1530, Rio De Janeiro, Brazil.

J

Jachowski, Lt. Leo A., Jr., 4011 Prospect St., Kensington, Md.

Jahnes, Capt. William G., MSC, 406th Medical General Lab., APO 500, % Postmaster, San Francisco, Calif.

Jamnbach, Hugo, Univ. of Massachusetts, Amherst, Mass.

Janes, Dr. Melvin J., Socony-Vacuum Labs., 412 Greenpoint Ave., Brooklyn 22, N. Y.

Janosko, Tim, 500 Patterson Ave., Stratford, Conn.

Jefe, de la Division de Entomologia y Zoologia, Apartado de Correos No. 4643, Maracay Edo Aragua, Venezuela

Jefferson, Raymond, 111 Beach St., Port Jefferson, N. Y.

Jenkins, Dale W., Med. Div., Army Chemical Center, Edgewood, Md.

Jenkins, R. D., J. & W. Exterminator Co., Bruce, Wisc.

Jensen, Dinnicmaud V., Nat'l Institutes of Health, Bethesda 14, Md.

Jobbins, D. Manley, N. J. Agric. Expt. Station, New Brunswick, N. J.

Johnson, Donald R., ECA Mission to Indonesia, Public Health Div., % American Embassy, Djakarta, Java, Indonesia

Johnson, Everett B., MCWA, USPHS, Louisiana St. Bd. Health, 511 Auburn Ave., Monroe, La.

Johnson, Nels J., 912 Pitner Ave., Evanston, Ill.

Jones, Alec C., Canadian Defence Research Bd., Ottawa, Ontario, Canada

Jones, G. Paul, Marin Co. Mosquito Abatement Dist., 24 Meadow Avenue, San Rafael, Calif.

Jones, Gilbert Fred, 2724 Waunona Way, Madison, Wisconsin

Jones, Dr. Jack C., Bldg. 5, Nat'l Inst. of Health, Bethesda 14, Md.

Josephson, Karl L., Supervisor, Box Elder Mosq. & Fly Ab. Dist., Brigham City, Utah

Joyce, Charles R., Public Health Service, P. O. Box 1410, Honolulu, T. H.

K

Kandouw, Dr. A. G. J., Chief, Malaria Control of East Indonesia, Macassar, Indonesia

Kane, Horace, 30 Jumel Pl., Saratoga Springs, N. Y.

KauLee, Foo, 466 Makaoe Lane, Honolulu 42, T. H.

- Kavaney, Vincent, Sr. San., City of Bismarck, 205 Cheyenne, Bismarck, N. Dak.
- Keener, George G., Jr., USPHS, Mitchell, Nebr.
- Keller, John C., U. S. Bur. Entom. USDA, Box 3391, Orlando, Florida
- Kemp, Robert L., Director, Manatee Co. Anti-Mosq. Control Board, P. O. Box 968, Bradenton, Florida
- Kerman, Paul D., President, The Drum Point Corp., 220 Southern Bldg., Washington, D. C.
- Kessler, J. C., 13th & Mediterranean Ave., Virginia Beach, Va.
- Ketcham, Tracey D., 80 Arnold Ave., West Babylon, N. Y.
- Kimball, Jack H., Orange Co. Mosq. Ab. Dist., P. O. Box 87, Santa Ana, Calif.
- King, Dr. Willard V., Bur. Ent. & Plant Quar. USDA, Box 3391, Orlando, Fla.
- Kinsey, LeRoy, 173 Ferncroft Road, Mineola, L. I., N. Y.
- Kirkland, Claude J., 92 Glenwood Ave., Leonia, N. J.
- Kirkpatrick, A. F., 111 Sutter St., San Francisco 4, Calif.
- Kittila, John, Supr., Nantucket Mosq. Con. Proj., 48 W. Chester St., Nantucket, Mass.
- Klassen, Clarence W., Illinois Dept. Pub. Health, State House, Springfield, Ill.
- Knight, Lt. Cmdr. Kenneth L., USN, Malaria & Mosquito Control, U. S. Naval Air Station Unit 1, Jacksonville, Fla.
- Knipe, Fred W., % Malaria Institute of India, 22 Alipore Road, Delhi 8, India
- Knipling, E. F., USDA, Bur. Ent. & Plant Quar., Room 4140, So. Agr. Bldg., Washington 25, D. C.
- Knote, Charles E., Cape Chemical Co., 33 N. Frederick St., Cape Girardeau, Mo.
- Knutson, Herbert, Univ. of Rhode Island, Kingston, R. I.
- Komp, W. H. W., U. S. Public Health Service, 6906 Dartmouth Ave., College Park, Md.
- Kotler, David C., 2270 N. Parkway, Memphis, Tenn.
- Koyen, Edward, Peltier Ave., Metuchen, N. J.
- Kramer, E. W., Eron Pest Control Inc., 817 Strong's Ave., Stephens Pt., Wisc.
- Kramer, Marvin C., 1413 12 St., Bismarck, North Dakota
- Krause, James B., Asst. Prof. Biol., University of Delaware, Newark, Delaware
- Krupa, John S., Bergen Co. Mosq. Comm., 353 Lanza Ave., Garfield, N. J.
- L
- LaBrecque, Germain C., USDA, P. O. Box 3391, Orlando, Florida
- La Ferla, Dr. Gaetano, Estacion de Malarologia, Coro, Falcon, Venezuela
- Lafferty, Boyd, Cape May Co. Mosq. Exterm. Comm., Cape May Court House, N. J.
- Langford, George S., Specialist of Insect Control, Dept. of Entomology, Univ. of Maryland, College Park, Maryland
- Lannon, Alvin J., Admin. Div. Entom., Dept. of Agr. & Cons., State House, Providence 2, R. I.
- Lassmann, Dr. Guenther W., Independencia #3, Jalapa, Veracruz, Mexico
- Lauret, Thomas H., P. O. Box 72, Sunnyvale, Calif.
- Lazarus, Edwin, Entom., Box 875, Langley Air Force Base, Va.
- Lea, Arden O., Jr., 2681 Brandon Road, Columbus 12, Ohio
- Leavens, W. T., Opr. Mgr., Leavens Bros. Air Serv. Ltd., Barker Airport, Toronto, Canada
- Leonard, Mortimer D., Entomologist, Julius Hyman & Co., 2480 16th St., N.W., Washington, D. C.
- Leslie, Jesse B., 390 Highwood Ave., Leonia, N. J.
- Lewis, Clarence W., Pres., Clarence W. Lewis & Son, Ltd., Crimsby, Ontario, Canada
- Librarian, London Sch. of Hyg. & Trop. Med., Keppel St., London WC1, England
- Lincicome, Dr. D. R., E. I. du Pont de Nemours Co., Vet. Chem. Res. Lab., R. D. 2, Newark, Del.
- Lindemann, Milton E., 2013 Harriet Ave., Minneapolis 18, Minn.
- Lindquist, A. W., Ent., Bur. Ent. & Plant Quar., USDA, Box 332, Corvallis, Ore.
- Lipes, Jack E., 2nd Lt., MSC, AUS, 547th P. Med. Surv. Det., APO 757, % PM, New York, N. Y.
- Lisciott, Frank R., USPHS, 120 Boylston St., Boston 16, Mass.
- Littig, Kent S., 439 Ansley St., Decatur, Georgia
- Little, Dr. Ralph B., West Windsor Township, Princeton, N. J.
- Livingston, Arnold, 57 Thirteenth St., Brooklyn, N. Y.
- Lloyd, Louis J., Asst. Supt., Cape Cod Mosq. Control Project, East Sandwich, Mass.
- Loeber, Thomas S., Lake Co. Mosq. Ab. Dist., Gen. Del., Lakeport, Calif.
- Logan, Bruce R., N. 5805 C St., Spokane, Wash.
- Long, John C., Tobacco By-Products & Chem. Corp., Box 415, Lancaster, Pa.
- Lopp, Oscar V., CDC Entomologist, State Health Dept., Montgomery, Ala.
- Lowe, Thomas, 822 Chestnut St., Roselle, N. J.
- Lowry, Walter R., HMC, USN, USN Air Facility, Navy 214, % FPO, New York, N. Y.
- Ludvik, G. F., Biology Section, Malaria Control Br. TVA, Wilson Dam, Ala.
- Lyman, F. Earle, Dept. of Zoology, South Illinois University, Carbondale, Ill.
- Lynch, E. E., Dade Co. Anti-Mosq. Dist., 905 Court House, Miami 32, Fla.
- Lynch, Thomas J., P. O. Box 55, Winnetka, Illinois
- Lyon, George C., Kempsville Mosq. Con. Comm., Sylvan Beach, Lynnhaven, Va.
- Mc
- McCauley, Robert H., USPHS, Pacific Northwest Drainage Basins, Bldg. 24, Swan Island, Portland 18, Ore.
- McChesney, Robert, Supt., Somerset Co. Mosq. Ext. Comm., Court House, Somerville, N. J.

McCoy, E. B., Kempsville Mosq. Con. Comm., 2905 E. Ocean View Ave., Norfolk, Va.

McDonald, A. F., Sales Mgr., The de Haviland Aircraft of Canada, Ltd., Postal Station L, Toronto, Ont., Canada

McDonald, Joseph, 4810 E. Princess Anne Rd., Norfolk 2, Va.

McDuffie, Wm. C., BEPQ, USDA, P. O. Box 3391, Orlando, Florida

McFealey, Otto, Pres., Desplaines Valley Mosq. Ab. Dist., 8130 Ogden Ave., Lyons, Ill.

McGowan, Thomas F., San Jose City Dept. Health, 285 S. E. Market St., San Jose, Calif.

McGuire, Carl L., Pest Control Section, P. M. Dept., U. S. Naval Station, Rodman, Canal Zone
McLeese, Roy W., 1248 Laird Ave., Salt Lake City, Utah

McLintock, J., Veterinary & Med. Entomology Unit, Div. of Entomology, Science Ser., Ottawa, Ontario, Canada

McMahon, P. J., McMahon Bros., 22 Edwards St., Binghamton, N. Y.

McNeel, T. E., PHS, Div. of Water Pollution Control, 519 Federal Bldg., Little Rock, Ark.

M

MacCreary, Donald, Univ. of Delaware, Newark, Del.

Macready, Samuel D., P. O. Box 1962, Cristobal, Canal Zone, Panama

Maddock, Mrs. Charles S., Jr., River Rd. Rd. 5, Trenton, N. J.

Maddock, Darrell R., Idaho St. Dept. Pub. Health, Rm. 322, State House, Boise, Idaho

Maddux, N. G., 146 Prospect Ave., Yardville Heights, N. J.

Mail, G. Allen, Pathfinder Irrigations Bldg., Mitchell, Neb.

Maine St. Dept. of Health & Wel., Div. of San. Engrg., State House, Augusta, Me.

Manager, Delano Mosq. Ab. Dist., Delano, Calif.

Manteufel, Lawrence A., Sewage Treatment Plant, City of Wausau, Wisc.

Mapes, Gordon W., Supt., Matadero Mosq. Ab. Dist., 310 University Ave., Rm. 202, Palo Alto, Calif.

Marcano, Dr. Antonio Gomez, Div. De Malariologia, Maracay, Aragua, Venezuela

Marchant, A. J., Pest Control Service Co., 1115 P St., Sacramento, Calif.

Marsh, Spencer S., Jr., 17 Fairview Ave., Madison, N. J.

Marsteller, C. H., USN, DPW, Administration Bldg., Norfolk Naval Operating Base, Va.

Martin, Guzman Garcia, Div. De Malariologia, Coro, Venezuela

Martin, T., 8130 Ogden Ave., Lyons, Ill.

Masters, Charles O., 4357 Jennings Road, Cleveland 9, Ohio

Matellus, Dr. George, Service De La Santa Publique, Port-Au-Prince, Haiti

May, W. Peyton, Bay Colony, Virginia Beach, Va.

Mead, Frank W., 2286 Indianola Ave., Columbus 2, Ohio

Mendicoa, Dr. Tomas, Carrera 8, No. 111, San Cristobal-Tachira, Venezuela

Merced Co. Mosq. Ab. Dist. Mgr., Merced, Calif.

Meyers, Ernest G., Bur. of Vector Control, 334 Rowell Bldg., Fresno, Calif.

Mikuni, Robert T., 840 Wiliwili St., Honolulu, T H

Miller, Dr. Albert, Associate Prof., Tulane, Dept., Tropical Medicine, 1430 Tulane Ave., New Orleans 12, La.

Millis, Phineas A., General Delivery, Accord, N. Y.

Minnich, V. Samuel, Box 4096, So. Daytona, Florida

Miralles, Antonio Jose, Hijo Estacion de Malariologia, Valencia, Venezuela

Moorefield, Herbert H., 303 Harker Hall, Univ. of Illinois, Urbana, Ill.

Moreno, Rafael Cabrera, Division de Malariologia, Maracay, Aragua, Venezuela

Morrill, Austin W., Jr., Sanit. Engr. Br., Erdl, Ft. Belvoir, Va.

Morris, J. C., County Manager, Warwick Co., Hilton Village, Va.

Mortenson, Earl W., Calif. St. Dep. Pub. Health, 334 Rowell Bldg., Fresno, Calif.

Mukaída, Tomio, 1651 Young St., Honolulu, T H

Mulhern, Thomas D., 334 Rowell Building, Fresno, Calif.

Mulrennan, John A., Entom., State Bd. Health, Box 210, Jacksonville, Fla.

Murdock, Lt. Wallace P., 520 South Fannin, Tyler, Texas

Murphy, Russel, Mercer Co. Mosq. Exterm. Comm., Court House, Trenton, N. J.

Murray, William S., Entom., U. S. Army Engineers, John H. Kerr Reservoir, South Hill, Va.

N

National Pest Control Assn., Ralph E. Heal, Tech. Dir., 30 Church St., New York 7, N. Y.

Neghme-Rodriguez, Dr. A., State University, P. O. Box 9183, Santiago, Chile

Neil, Charles G., 340 Grand St., Paterson, N. J.

Nelson, Dr. F. C., Entomologist, Esso Standard Oil Co., Insecticide Laboratories, Box 222, Linden, N. J.

Nelson, James A., Editor, Pest Control Magazine, 1900 Euclid Bldg., Cleveland 15, Ohio

Newlin, Dr. Theodore A., Morris Co. Mosq. Ext. Comm., Woodland Ave., Rockaway, N. J.

Newman, Paul C., Morris Co. Mosq. Ext. Comm., Morris Plains, N. J.

Nichols, Delore, Sec., Davis Co. Mosq. Con. Comm., Farmington, Utah

Niebanck, Louis, Jr., 31 Lee Ave., Rockville Center, N. Y.

Nielsen, Lewis T., Dept. Zoology & Entom., Univ. of Utah, Salt Lake City, Utah

Nieto-Caicedo, Miguel, M.D., Division De Malariologia, Trujillo, Venezuela

Northern San Joaquin Co. M.A.D. President, 404 West Pine St., Lodi, Calif.

Nussbaum, Myron G., 2664 N. Clark St., Chicago 14, Ill.

O

Oberst, Fred, Mgr., Abalene Pest Con. Serv. Inc., 30 Duprey St., Saranac Lake, N. Y.

Ochoa, Sal, Chief Insp., Kern Mosq. Ab. Dist., 1118 1st St., Bakersfield, Calif.

Officer in Charge, Veterinary & Medical Ent. Sec. Science Ser., Canada Agri., P. O. Box 210, Kamloops, B. C., Canada

Olinger, Lee D., 2nd Lt. USAF, Office of the Air Surgeon, HQ Second Air Force, Barksdale Air Force Base, La.

Owen, Dr. W. B., Prof. Zoology, Univ. of Wyoming, Laramie, Wyo.

P

Page, A. P., Jr., Portsmouth Mos. Con. Comm., 618 Riverview Ave., Portsmouth, Va.

Page, Robert Zane, Director, Pest Control Div., Dist. Pub. Wks. Officer, 5th Naval Dist., 8116 Lion Ave., Norfolk 3, Va.

Pal, Dr. Rajindar, M.Sc., Malaria Inst. of India, 22 Alipore Road, Delhi, India

Parrish, Dale W., P. O. Box 983, Cocoa, Florida
Pepper, Dr. B. B., N. J. Agr. Expt. Sta., New Brunswick, N. J.

Perine, Keble B., Chairman, Belmont Health Dept., Town Hall Annex, Belmont 78, Mass.

Perry, William J., M.D., 8 York Court, York Road, Baltimore 18, Md.

Peters, H. D., Dir., Div. San. Dept. Pub. Health, 940 Main St., Jacksonville, Fla.

Peters, Richard F., Rt. 3, Box 1763, Springhill Rd., Lafayette, Calif.

Peters, Robert H., No. San Joaquin Mos. Ab. Dist., 404 W. Pine St., Lodi, Calif.

Peterson, Bob V., Dept. of Biology, Div. of Inv. Zool. & Ent., Room 304, University of Utah, Salt Lake City 1, Utah

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Peterson, Douglas G., Div. of Entomology, 370 Science Service Bldg., Carling Ave., Ottawa, Ontario, Canada

Peterson, Dr. P. D., Tech. Dir. Agric. Sales, Stauffer Chem. Co., 420 Lexington Ave., New York, N. Y.

Petrakis, Capt. M. M., 0533338 MSC, ORC, 6th Malaria Survey Det., APO 503 % Postmaster, San Francisco, Calif.

Pierce, Louis B., Mercer Co. Mosq. Exterm. Comm., Court House, Trenton, N. J.

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Power, A. E., Box 222, Moncks Corner, S. C.

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Q

Quartermann, Kenneth D., USPHS, P. O. Box 192, Savannah Beach, Ga.

R

Rachou, Rene G., National Malaria Service, Rua Laranjeiras 550 ap. 1306, Rio de Janeiro, Brazil
Rafle, Dr. Philip J., Commr., Dept. of Public Health, Riverhead, L. I., N. Y.

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Richards, Louis J., 1077 E. Jersey St., Elizabeth, N. J.

Ritter, Anderson B., Chief, Sect. of Insect Vector Control, Louisiana State Health Dept., 936 Broadway, New Orleans 18, La.

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S

Safrenck, Arthur Paul, Senior Inspector, Bergen Co. Mosq. Comm., 109 Nicholas Ave., Little Ferry, N. J.

Sameth, J. Edwin, Gen. Mgr., Western Exterm Co. Inc., 1060 Broad St., Newark 2, N. J.

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Schuh, Joe, 4039 Shasta Way, Klamath Falls, Oregon

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Scudder, Dr. Harvey I., Public Health Service, Vector Control Field Station, 5405 E. Shields Ave., Fresno 3, Calif.

Scabrook, Edwin L., Palm Beach Co. Anti-Mosquito, Court House Annex, West Palm Beach, Fla.

Senior-White, Ronald A., P. O. Box 556, Port of Spain, Trinidad, B. W. I.

Shambaugh, George F., 2995 Trabus Road, Columbus 12, Ohio

Shanafelt, John G., Jr., 13641 Chapman Ave., Route 2, Orange, Calif.

Shanahan, Edw. J., NSMAD, 2023 Schiller Ave., Wilmette, Ill.

Sharp, John F., Asst. Entomologist, Div. of Entomology, Room 370, Science Serv. Bldg., Ottawa, Ontario, Canada

Sheppard, Raymond E., P. O. Box 1888, Sarasota, Fla.

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Socparmo, H. T., Chief, Malaria Control, Djalan Diponegoro 69, Djarkarta, Indonesia

Sollers, Helen, Bur. Ent. & Plant Quar. USDA, Washington 25, D. C.

Soper, Dr. Fred L., 204 Rosemary St., Chevy Chase, Md.

Soroker, Robert H., Bur. of Vector Control, 5701 Morena Way, Sacramento 20, Calif.

So. Carolina State Bd. of Health, Div. of Disease Control, Wade Hampton Office Bldg., Columbia, S. C.

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Stahler, Nathan, Naval Med. Res. Inst., Bethesda 14, Md.

Stallman, M. M., 15 Summit St., East Orange, N. J.

Stansfield, Edmund J., Greater Winnipeg Anti-Mosq. Campaign, 917 Riverwood Ave., Fort Gerry, Manitoba, Canada

Stapp, Capt. Robert R., Jr., 0-395418 Ryukyus Army Hospital, 8114 Army Unit, APO 719, San Francisco, Calif.

Stauffer, George F., Box 70, Sharon Hill, Pa.

Stearns, Lt. Col. L. A., 278 Orchard Road, Newark, Delaware

Steiner, Henry, Abash Insect Control Service, P. O. Box 1027, McAllen, Tex.

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Sweeney, Charles F., Park Commission, City of Fitchburg, Mass.

T

Taylor, Blaine C., Calif. Spray Chem. Corp., P. O. Box 2396, Salt Lake City, Utah

Taylor, F., 8130 Ogden Ave., Lyons, Ill.

Taylor, John E., USPHS, Ark. State Board of Health, State Health Bldg., Little Rock, Ark.

Taylor, John W., Pilot, Tech. Dev. Div. USPHS, Box 769, Savannah, Ga.

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Trembley, Miss Helen L., Editor of Reviews Mosq. News, % Nat. Inst. of Health, Bethesda 14, Md.

Tulloch, G. S., Dept. Biol., Brooklyn College, Bedford Ave. & Ave. H, Brooklyn 10, N. Y.

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Twinn, Roy L., 329 Fifth Ave., Ottawa, Ontario, Canada

U

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V

Valderrama, Dr. M. Prada, Div. de Malaria-ologia, Av. 2A, Las Flores 17, Pte. Hierro, Caracas, Venezuela

Van Aelstyn, Edw. L., M.D., Health Officer, P. O. Box 149, Vancouver, Wash.

Van Brederode, Hendrik, 311 Godwin Ave., Midland Park, N. J.

Van Derwerker, Ralph J., Senior Sanitary Engineer, U. S. Public Health Service, Washington 25, D. C.

Van Hovenberg, H. W., P. O. Box 288, Mt. Pleasant, Tex.

Vannote, Robert L., 3 Franklin Pl., Morris Plains, N. J.

Vargas, Dr. Luis, Apartado Postal 19205, Mexico, D. F.

Vazquez, Dr. Angel Diaz, Division de Malaria-ologia, Maracay, Aragua, Venezuela

Vercival, Archibald, P. O. Box 503, Port Arthur, Ontario, Canada

Vicente, Dr. Jose Blazquez, Apartado 646, Maracay, Aragua, Venezuela

Vickers, Mrs. Emma M., Atl. Co. Mos. Comm., 5 S. Virginia Ave., Atlantic City, N. J.

Vincent, Rufus H., 5608 37th Ave., Hyattsville, Md.

Vitanza, Francesco, Calle Carvajal No. 7, Maturin, Venezuela

Vogt, George B., Div. Insects, U. S. Nat'l Museum, Washington 25, D. C.

W

Walker, David W., APO 928, MSA, STEM, % Postmaster, San Francisco, Calif.

Walker, John R., Calif. Dept. Pub. Health, 2180 Milvia St., Berkeley 4, Calif.

Walker, Walter, Pres., Accurate Tool Co., 15 Manufacturers Place, Newark 5, N. J.

Wallace, Dr. R. B., Malayan Amer. Plant Ltd., Chartered Bank Chambers, Penang, Malaya

Wallis, Robert C., Johns Hopkins Sch. of Pub. H., 615 N. Wolf St., Baltimore 5, Md.

Walsh, Rev. Joseph E., 9 Rosedale Ave., Morris Plains, N. J.

Warner, Wm. P., USPHS, CDC, % Bur. of Vector Control, 2180 Milvia St., Berkeley 4, Calif.

Warnock, Ward, Co. San., Farmington, Utah
Washburn, G. Edwin, Turlock Mosq. Ab. Dist., P. O. Box 629, Turlock, Calif.

Weathersby, Lt. Cdr. A. B., MSC, USN, Naval Medical Research Inst., National Naval Med. Center, Bethesda 14, Md.

Webb, Lt. Col. J. E., Jr., 0-29271, 4th Army Area Med. Lab., BAMC, Fort Sam Houston, Tex.

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Weiss, A. M., Pres., All Westchester Exter. Corp., 165 E. Third St., Mount Vernon, N. Y.

Weldon, George H., Velsicol Corp., 2082 Center St., Berkeley, Calif.

West, Allen S., Queens University, Kingston, Ontario, Canada

Weyer, Albert E., CIA, Bananera de Costa Rica, Golfito, Costa Rica

White, Orville E., Sr., Director, Municipal Pest Cont., Rt. 1, Box 916, Ft. Lauderdale, Florida

Whitehead, Dr. F. E., Univ. of Arkansas, Fayetteville, Ark.

Wilkins, Robert A., Supt., Salt Lake City Mosq. Ab. Dist., 401 City & County Bldg., Salt Lake City, Utah

Williams, Donald, Chief Electrical Engineer, South Porto Rico Sugar Co., P. O. Box 809, Ensenada, Puerto Rico

Williams, J. Melton, Dir. Mosquito Control, Court House Bldg., Sarasota, Fla.

Williams, Michele, 310 D St., N.E., Washington, D. C.

Williams, Dr. Roger W., Med. Ent., Sch. Pub. Health, Columbia U., 600 W. 168 St., New York 32, N. Y.

Williamson, C. T., 16 Orowoc Ave., Islip, N. Y.

Wilson, Albert William, Dept. of Public Health, 57 Murray St., Perth, W. Australia

Wilson, C. A., Dept. of Zool. & Ent., Miss. State College, State College, Miss.

Wilson, Charles S., USPHS, Box 960, Anchorage, Alaska

Wilson, James W., Pres., Custom Fogging Inc., 8439 Burthe St., New Orleans, La.

Wilson, Roy J., P. O. Box 3026, Port Arthur, Texas

Wisecup, C. B., Rep. & Utilities Div., 2nd Army Engineer, Ft. Meade, Md.

Wood, Frank B., Civil Engineer, San. Section, So. Atlantic Div. Corp. of Eng., P. O. Box 1889, Atlanta, Georgia

Woodbury, Dr. E. N., Entomologist, Hercules Powder Co., 312 Beneficial Loan Bldg., 13th & Market Sts., Wilmington, Delaware

Wray, Franklin C., Desplaines Valley Mosq. Ab. Dist., 8130 Ogden Ave., Lyons, Ill.

Wright, Percy M., San. Insp., Bermuda Health Dept., Witu Warick, E. Bermuda

Wymer, William H., P. O. Box 426, Balboa, Canal Zone

Y

Yamaguchi, Tiyo, 2132 Ivy St., Fresno, Calif.

Yamamoto, Willard C., 2919 I Kolowalu St., Honolulu, T. H.

Yates, Willard W., Bur. Ento. & Plant Quar. USDA, Box 332, Corvallis, Ore.

Young, Orson Whitney, Weber College, Ogden, Utah

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Z

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B

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Chief, Division of Entomology, Department of Agriculture, Science Service Bldg., Ottawa, Canada

Chief, Water Projects Section, CDC Activities, USPHS, Box 386, Salt Lake City, Utah

Chipman Chemical Co. Inc., Box 679, Palo Alto, Calif.

Chow, C. Y., WHO, Field Training for Study & Control of Insect-Borne Diseases, Kurunegala, Ceylon

Coachella Mosquito Abatement District, Public Health Center, Patton Court, Indio, Calif.

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